University of Alberta

Inactivation of *Giardia muris* Cysts in Water Using Multiple Disinfectants

by

James Stuart Bradbury ©

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Abstract

The production of safe drinking water involves reduction of microbiological threats as well as reduction in potentially health threatening disinfection byproducts. The challenge faced by the water treatment industry requires a balance between increasing disinfectant levels to reduce biological threats from protozoan cysts or oocysts and reducing disinfectant levels to minimize levels of disinfection byproducts. This study demonstrates increased levels of *Giardia muris* cyst inactivation resulted from sequential application of two different disinfectants, when compared to the sum of inactivations expected from single dose applications.

Batch reactor experiments were conducted in oxidant demand free phosphate buffered laboratory water at 5°C. Cyst inactivations were measured by *in vitro* excystation. Significant synergistic effects resulted from sequential application of the following pairs of disinfectants: chlorine then monochloramine at pH 8; chlorine dioxide then chlorine at pH 6 and 8; ozone then chlorine at pH 6 and 8; ozone then monochloramine at pH 8 and 11.

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1. Introduction

Today's water treatment industry continues to face difficult challenges. For example, on one side, there is an increasing number of microorganisms found in source waters that are identified as pathogenic, some of which are highly resistant to current disinfection practices. On the other side, toxicological and epidemiological studies suggest that disinfection by-products may have adverse health effects. The goal of this study was to study an improved method of disinfection on a model organism of interest to the water industry.

The primary objective of this research was to determine if there is a synergism between disinfectants when inactivating *Giardia muris* cysts. Advancing the knowledge base on possible interactions between disinfectants applied sequentially, may lead to acceptable levels of pathogen inactivation with reductions in the required disinfectants Ct values. A secondary objective was to demonstrate the effect of pH on cyst inactivation following application of multiple disinfectants.

Combinations investigated were: chlorine - monochloramine at pH 8 and 11; chlorine dioxide - chlorine at pH 6 and 8; chlorine dioxide - monochloramine at pH 8 and 11; ozone - chlorine at pH 6, 8 and 11; and ozone - monochloramine at pH 8 and 11. This investigation involved eleven sequential experiments plus sufficient individual dose response experiments to establish expected inactivation levels from single disinfectant doses. All sequential experiments and the respective individual dose experiments were replicated at least once and conducted in a random manner. A synergistic effect was

demonstrated when the net inactivation from a sequential test is statistically greater than the sum of expected inactivation resulting from the respective single disinfectant tests.

All experiments were conducted at bench scale using 250 mL Erlenmeyer flask batch reactors with 200 mL of 5°C laboratory water. Laboratory waters were made oxidant demand free and phosphate buffered at pH 6, 8 or 11. Inactivations were measured by *in vitro* excystation.

2. Literature Review

Recent outbreaks of waterborne disease in industrialized societies are attributed to protozoan pathogens, specifically *Giardia lamblia* and *Cryptosporidium parvum*. Typically, a water treatment plant will rely on a combination of coagulation with sedimentation, filtration and disinfection steps to provide an acceptable level of protection against waterborne protozoan infections. The United States Environmental Protection Agency (USEPA) is considering amendments to the Surface Water Treatment Rule (SWTR) for improved control of *G. lamblia*, *C. parvum* and viruses. The SWTR regulation currently regulates *G. lamblia* to a minimum of three log units of combined inactivation and removal (Pontius 1990). Compliance with the SWTR is assessed by allowing inactivation log credits for treatment technology techniques, turbidity limitations and minimum residual disinfectant levels in the distribution system. If a filtration stage is not included in the treatment train then regulations on eight criteria must be met. The first criteria is coliform limits of less than or equal to 20 per 100 mL in 90 % of the samples for fecal coliforms or 100 per 100 mL in 90 % of the samples for total coliforms. The second criteria is a turbidity limit of less than 5 ntu measured every four hours. The third criteria

is disinfection in which the utility must demonstrate CT values sufficient for three and four log inactivation of *Giardia* cysts and viruses respectively. Included with this criteria is a requirement of 0.2 mg/L disinfectant residual entering the distribution system with the provision that the disinfectant residual cannot be non detectable in more than 5 % of samples per month. The remaining five criteria are, a watershed control program, on-site facility inspection by state or authorized third party, absence of waterborne disease outbreaks in serviced community and compliance with total trihalomethane contaminant level and the total coliform rule.

Often a water treatment plant will use turbidity removal as an indicator of cyst removal. However pilot plant studies by Logsdon *et al.* (1985) indicated *Giardia* cyst levels of 3 to 10 cysts per liter in the effluent of properly operating filters and that cyst concentration increased substantially with a corresponding small increase in turbidity. In conclusion, Logsdon *et al.* (1985) stated that effective control of *Giardia* cysts results from correct application of sedimentation, filtration and disinfection. In a later study, LeChevallier *et al.* (1992) reported a significant correlation between particle count reduction and parasite removal, and also that particle counts were sensitive to small changes in filter performance. However, these authors also cautioned that parasite removal efficiency may be dependent on source water, thus the relationship between particle counts and parasite removal is likely to be different for other plants. LeChevallier *et al.* (1991) demonstrated that treatment plants with high levels of *Giardia* cysts and *Cryptosporidium* oocysts in the raw water are more likely to detect cysts or oocysts in finished water. LeChevallier *et al.* (1991) further reported that average treatment plants may require a five log reduction to ensure an annual infection risk level less than 1:10,000

and also that oocyst and cyst reductions of 2.2 to 2.5 log may be expected through clarification and filtration which leaves a potential of 2.5 log inactivation to be met by disinfection . A five log reduction requirement would occur when treating a source water containing 70 *Giardia* cysts per 100 liters of water (Rose *et al.* 1991). Many water treatment plants will meet this disinfection requirement by applying a first disinfectant dose before filtration (either in the raw water or in settled water) and a second disinfectant dose following filtration. The first disinfectant dose is typically free chlorine. The second disinfectant is needed to provide an acceptable residual throughout the distribution system and is typically monochloramine or free chlorine. Ozone and chlorine dioxide are alternates for free chlorine and may be used as raw or settled water disinfectants.

The interactive effect on pathogen inactivation resulting from this sequential application of alternate disinfectants has not been well characterized. Bryant *et al.* (1992) present the following formulae which may used to calculate the expected total percentage inactivation resulting from sequential disinfection steps:

$$G_{t,n} = G_{t,n-1} + \left(\frac{G_n \times \left(100 - G_{t,n-1}\right)}{100} \right) \qquad \textbf{Equation 1}$$

Where:

n = number of points of pathogen removal or disinfection;

G_n = percent inactivation achieved by point n;

$G_{t,n}$ = expected total percent inactivation achieved by n points;

$G_{t,n-1}$ = total percent inactivation achieved after n-1 points.

Alternatively, an expected log inactivation ratio may be obtained by summing the expected log inactivation ratios for each point of disinfectant application as follows:

$$Log_{t,n} = \sum_{n=1}^{n} Log_n\left(\frac{N}{N_o}\right) \qquad \textbf{Equation 2}$$

where:

n = number of disinfection points;

$Log_{t,n}$ = expected Total Log inactivation ratio achieved by n points applied sequentially;

Log_n = log inactivation ratio achieved for each point n alone;

N = percent of surviving organisms at point n;

N_o = percent of viable organisms prior to disinfection step n.

Either of these methods yield the same result and assume multiple disinfectant results are strictly additive. Thus, synergism may be defined as a final sequential log inactivation which is greater than the sum of expected individual inactivations. Antagonism may be defined as a final sequential log inactivation which is less than the sum of expected individual inactivations.

Waterborne Pathogens

Disinfection remains a vital step in public protection from pathogens present in water used for drinking, washing and irrigation of food products (Sobsey 1989). Recent disease outbreaks in industrialized societies serve as potent reminders of the acute need for effective disinfection of water. *Cryptosporidium parvum* contaminated drinking water in Milwaukee Wisconsin in 1993 resulted in greater than 300,000 illnesses (Edwards 1993).

and in Kelowna British Columbia in 1995 resulted in an estimated 10,000 illnesses (CP 1996). *Cyclospora cayetanensis* on strawberries irrigated with contaminated water resulted in a reported 35 illnesses in Toronto, Ontario, (Immen 1996). *Vibrio cholerae* in contaminated water resulted in over 300,000 illness in South America in 1991 (Diamond 1992). As recently as 1992, Craun (1992) reported that half of the reported waterborne disease outbreaks are of undetermined etiology. Work by Payment *et al.* (1991), indicates that there is an unreported nontrivial endemic level of illness resulting from consumption of tap water conventionally treated to current standards. To arrive at this conclusion, Payment *et al.* (1991) conducted a randomized study to compare incidence rates of gastrointestinal illness between two groups of people. The first group drank unmodified tap water, while the second group drank tap water passed through an under the sink commercial domestic reverse osmosis filtration unit. These persistent incidents and threat of illness from drinking water in industrialized societies illustrates the need for advancing the knowledge base of disinfection technologies.

Microbiological infectious agents potentially present in a source water fall into four biological categories: bacteria, protozoa, viruses and helminths (James M. Montgomery Consulting Engineers 1985). Bacteria are non photosynthetic simple unicellular organisms comprised of a single exterior membrane enclosing cytoplasm and a nucleus. Many bacteria are motile, reproduce by binary fission and range in size from 0.1 to 10 μm long. Typical bacterial pathogenic microorganisms of water treatment concern are listed in Table 1.

Protozoa are non photosynthetic complex unicellular organisms, containing: a membrane enclosed nucleus, cytoplasm and a variety of specialized organelles. Most protozoa are motile by cilia, flagella or amoeboid locomotion, reproduce by binary fission and range in length from 8 to 100 μm. Many pathogenic protozoa are capable of forming an inert cyst or oocyst for survival in the external environment. Typical protozoan pathogenic microorganisms of water treatment concern are also listed in Table 1.

Viruses are the simplest and smallest of the microbiological pathogens. A virus consists of a strand of nucleic acid surrounded by a protein capsid typically 25 to 100 nm in length. Typical viral pathogenic microorganisms of water treatment concern are listed in Table 1.

Helminths are parasitic worms which reproduce through eggs (James M. Montgomery Consulting Engineers 1985). Helminth classifications includes flukes, tapeworms and nematodes which are contacted through water contaminated with eggs, larvae or adult worms depending on which life cycle stage of a particular species exists in drinking water sources. Generally helminths are not a widespread concern in drinking water treatment in Canada and the United States (James M. Montgomery Consulting Engineers 1985).

Table 1 Typical human pathogens of water treatment concern

Bacterial

Salmonella typhi
other Salmonella spp.
Shigella spp.
- *S. sonnei*
- *S. flexnerni*
- *S. dysenteria*
- *S. boydii*

Vibrio cholerae
Yersinia enterolitica
Clostridiuum perfringens
Legionella pneumophila
Aeromonas spp.

Protozoan

Giardia lamblia
Cryptosporidium parvum

Entamoeba histolytica

Viral

Enteroviruses:
- *Polioviruses*
- *Coxsackie viruses A*
- *Coxsackie viruses B*
- *Echoviruses*
- other enteroviruses

Other Viruses
- *Adenoviruses*
- *Rotaviruses*
- *Norwak agent*
- *Hepatitus A*

Note: partial listing of data presented by (James M. Montgomery Consulting Engineers 1985)

Giardia Species

Giardia species of protozoa are binucleate flagellated parasites which are represented by three morphological species (Adam 1991):

- *G. agilis* which infect amphibians;
- *G. muris* which infect rodents, birds and reptiles; and
- *G. lamblia* (also called *G. duodenalis* or *G. intestinalis*) which infect mammals (humans), birds and reptiles.

Giardia species follow a simple life cycle. *Giardia* exist as trophozoites inside the small intestine, reproduce by binary fission, have four pairs of flagella for motility and possibly other functions, and have a ventral disk for attachment to the intestinal mucosa of the host. Trophozoites contained in the luminal flow will encyst and both nuclei divide simultaneously. Thus, a mature *G. muris* cyst will contain a trophozoite with an arrested division after completing karyokinesis but before completing cytokinesis (Schaefer 1990). The newly formed cyst is the inert and environmentally resistant intra host stage found in surface waters. Once the cyst is ingested by the host, excystation begins within five to 10 min. of arrival into appropriate conditions (Adam 1991). After the trophozoite pair have exited the cyst shell, cell division resumes to yield two binucleate trophozoites. Cysts released into the external aquatic environment may remain viable for several months (deRegnier *et al.* (1989). In the deRegnier study, the authors demonstrated *G. muris* cyst viability over two to three months for cysts contained in the fecal biomass and suspended in low temperature lake and river waters.

Giardia Presence in Source Waters

The widespread presence of *Giardia* and *Cryptosporidium* in surface raw water sources was demonstrated in two studies on the presence of *Giardia* and *Cryptosporidium* in raw and finished water. The first study presented surface water treatment plant data collected from 66 plants during 1988 to 1990 (LeChevallier, Norton et al. 1991). From this study reported occurrence results show *Giardia* spp. at 81 %, *Cryptosporidium* spp. at 87 % of samples tested and 97 % of the samples contained at least one of the two pathogens. The second study presented surface water treatment plant data collected from 72 plants during 1991 to 1993 and included treatment plants from the initial study (LeChevallier and Norton 1995). Reported occurrence results from this study show *Giardia* spp. at 45 %, *Cryptosporidium* spp. at 51.5 % of samples tested and 70 % of the samples contained at least one of the two pathogens. LeChevallier and Norton (1995) summarized the occurrence range of *Giardia* spp. as 40 to 60 % of the samples and *Cryptosporidium* spp. as 60 to 80 % of the samples and one protected watershed site was consistently negative for either cysts or oocysts.

LeChevallier *et al.* (1995) suggested the possibility of a cyclic phenomenon in cyst and oocyst concentration levels in surface water sources. In view of this LeChevallier *et al.* (1995) recommended that a utility establish a data base of cyst and oocyst presence which covers a prolonged period of time instead of intense sampling over a short period of time. Once a data base including cyst and oocyst concentrations plus particle counts is compiled for raw, settled and filtered water then an effective overall treatment goal may be established. LeChevallier *et al.* (1995) further reported that once cyst and oocyst

reductions are correlated with particle removals then the disinfection requirement may be determined by the difference between treatment goal and reductions due to particle removal.

Required Level of Protection Against Giardiasis

Bennett *et al.* present an analysis of data compiled in 1985, to assess the negative impact of infectious disease, by: the United States National Center for Health Statistics, Center for Infectious Diseases, Center for Prevention Services and Centers for Disease Control (Bennett, Holmberg et al. 1987). This study suggested that infectious diseases in the United States resulted in:

- two million years of life lost before age sixty five;
- fifty two million hospital days;
- almost two billion days lost from work, school and other major activities; and
- costs of more than seventeen billion dollars annually (excluding costs of death, lost wages, loss of productivity, reactions to treatment or other indirect costs).

Included in these conclusions was the impact of enteric infections which accounted for nine million hospital days with direct costs of three billion dollars annually. The authors claimed in summary that substantial infection reductions are possible using existing and soon to be available interventions. The USEPA used the data compiled by Bennett *et al.* on death and incidence of illness caused by waterborne infectious agents, to develop an estimated annual risk of waterborne illness and lifetime risk of death at 4×10^{-3} and 3×10^{-4}, respectively as explained through the federal register (1994). Using these values the USEPA (1994) then determined a health risk goal of less than 10^{-4} infections

annually (one *G. lamblia* infection per 10,000 exposed persons), with the consequent benefit of higher levels of protection against viruses and bacteria.

A description of the simple exponential model developed for estimating risk of infection following waterborne exposure to *Giardia* cysts is presented by Rose *et al.* (1991). Rose *et al.* (1991) followed the approach used by Haas (1983) to evaluate assessment models and determined the simple exponential model to be statistically consistent when applied to the Rendtorff data. The Rendtorff data are results of a human infectivity study using one strain of *G. lamblia* and a relatively small sample population of volunteers (Rendtorff 1954; Rendtorff and Holt 1954). The following simple exponential models were used to determine risk of infection resulting from exposure to *G. lamblia* Rose *et al.* (1991):

$$\text{daily risk} = 1-\exp(-rN) \qquad \textbf{Equation 3}$$

and

$$\text{annual risk} = 1-(1-\exp(-rN))^{x} \qquad \textbf{Equation 4}$$

where:

N = average number of cysts exposed to for daily risk and geometric average number of cysts for annual risk;

r = 0.01982 (95 % confidence region 0.009798 to 0.03582) = fraction of microorganisms which survive to initiate infection;

x = number of days exposed.

Determination of an acceptable waterborne cyst concentration was achieved by substituting 10^{-4} for the risk level and assuming two liters of water consumed per person per day. Calculated allowable levels were 0.25 cysts per 100 L for daily average exposure

and 7×10^{-4} cysts per 100 L daily exposure for an annual risk of infection (Rose, Haas et al. 1991). Therefore a one log reduction in either cyst concentration or viability is required for each tenfold increase in cyst concentration above the allowable level calculated for a 10^{-4} risk.

Giardia muris as a Model for *Giardia lamblia*

Disinfection studies involving *G. muris* and *G. lamblia* typically employ *in vitro* excystation to measure kills less than 3 log units. Measurement of higher levels requires the use of animal infectivity models, such as the mouse model for *G. muris* (Roberts-Thomson, Stevens et al. 1976; Belosevic and Faubert 1983) or the Mongolian gerbil model for *G. lamblia* (Belosevic, Faubert et al. 1983).

Disinfection studies often use *Giardia muris* and excystation when measuring disinfection inactivation less than 2.5 to 3.0 log units for the following reasons (Schaefer 1990):

- *G. muris* is not a human pathogen;
- *G. muris* cyst release in CD-1 Swiss mice is continuous, whereas *G. lamblia* cyst passage in Mongolian gerbils is intermittent;
- *G. muris* cysts can routinely be excysted at levels greater than 90 percent, while excystation of *G. lamblia* cysts is erratic and often less than 90 percent;
- Animal infectivity models require one to two weeks to conduct and incur additional expenses of animal procurement, care and housing;
- Excystation procedures may be completed within three to four hours and typically require a minimum of 10^5 cysts (Schaefer 1990); and

- Empty cyst walls are readily visible using phase contrast microscopy while *G. lamblia* empty cyst walls are difficult to see.

Percent excystation *for G lamblia* and *G. muris* are typically calculated differently due to the difficulty of seeing *G. lamblia* empty cyst walls. Excystation of *G. lamblia* is calculated from:

$$\frac{TET/2+PET}{TET/2+PET+IC}\times 100\% \qquad \textbf{Equation 5}$$

where:

PET = partially excysted trophozoites

TET = totally excysted trophozoites

IC = intact cysts

Excystation of *G. muris* is calculated from:

$$\frac{ECW+PET}{ECW+PET+IC}\times 100\% \qquad \textbf{Equation 6}$$

where:

ECW = empty cyst wall

Additional methods of viability determination, are either used infrequently or are under evaluation and include vital dye exclusion, fluorogenic dye staining, animal infectivity and morphological examination (Schaefer 1990).

Detection Methods for *Giardia lamblia* and *Cryptosporidium parvum*

Papers by Rochelle *et al.* (Rochelle, De Leon et al. 1995) and Jakubowski *et al.* (Jakubowski, Butros et al. 1996) summarize available and developing technologies for detecting *Giardia* species in water. Methods presented are summarized in Table 2.

Table 2, Detection methods for *Giardia* and *Cryptosporidium* in water

Method	Description
IFA (Indirect Immunofluorescence Assay)	Cysts are collected and purified by filtration and centrifugation, then tagged with: anti-*Giardia* primary antibodies followed by a fluorescein isothiocyanate anti body. Cyst concentration is assessed by fluorescence microscopy.
ELISA (Enzyme Linked Immunosorbent Assay)	Typically applied directly to stool samples or purified concentrated samples. Utilizes colour developed by an anti body - antigen reaction with an enzyme and substrate. Requires use of a microscope or a microplate reader
UV-VIS (Ultraviolet-visible Spectroscopy)	Requires concentration and purification by filtration and centrifugation. Visible range used to determine size and quantity, while the ultra violet region may be used to identify specific organisms. Use of fluorescein isothiocyanate anti bodies enhances technique sensitivity and use of fluorogenic vital dyes may enable this method to be used for viability assays. This method is under development.
Electrorotation	Potential process for *Giardia* whereby cysts are attached to anti-body coated metal beads and placed in a rotating electric field. This method is developmental and offers the potential to measure viability by differences in rotation direction between live and dead cysts.
Flow cytometry	Concentrated and purified samples are stained with fluorescein isothiocyanate anti bodies, the sample is then analyzed by the flow cytometer for predefined size and fluorescence. Microscopic examination is required for confirmation of cyst identification. Currently used in Europe for cyst enumeration and has potential for viability measurement.

Table 2, Continued

Method	Description
PCR (Polymerase Chain Reaction)	This method is under development and is based on extraction of cell DNA, amplification, hybridization and analysis by gel electrophoresis.
Cell culture	Potential alternative to animal infectivity models as a measure of infectivity using *in vitro* cell cultivation. This method is in the developmental stage.
Immunomagnetic Separation	Method whereby relevant antibodies are immobilized onto magnetic beads are added to a concentrated and purified sample. Following an incubation period cysts or oocysts are recovered using a magnet. Once captured cysts or oocysts may be detected by another procedure. This method is under development.

Note: compiled from Rochelle *et al.* (Rochelle, De Leon et al. 1995) and Jakubowski *et al.* (Jakubowski, Butros et al. 1996)

Chemistry of Disinfectants

Chlorine Dioxide

Aieta *et al.* (1986), reviewed the use of chlorine dioxide in drinking water treatment and presented the following reasons for onsite generation: chlorine dioxide vapour at 40 KPa above atmospheric pressure is potentially explosive; solutions containing less than 10 mg/L will not generate sufficient vapour pressure to be a hazard; and chlorine dioxide exists in solution as a dissolved gas.

In drinking water treatment sodium chlorite is typically the raw material of choice for chlorine dioxide production when high purity is required in quantities less than 2000 kg/day. Generation of chlorine dioxide using sodium chlorite can be achieved by several methods (Kieffer and Gordon 1968; Kaczur and Cawlfield 1993). The most commonly used methods in drinking water treatment are aqueous chlorine sodium chlorite system and the gas chlorine sodium chlorite system (1986). The oxidative process involves a reaction of chlorite with added chlorine or hypochlorous acid, generally at a pH of 2 to 5. Both methods produce chlorine dioxide as a result of chlorous acid formation from chlorite and hydrogen followed by subsequent reaction with a chlorine species ($HOCl$, Cl^-, Cl_2) to produce dichlorine dioxide which in turn dissociates to produce chlorine dioxide. At pH 5 chlorous acid content is less than 0.2 % thus chlorine dioxide production becomes insignificant and the chlorite reaction with chlorine and hypochlorous acid to produce chlorate becomes dominant (Kieffer and Gordon 1968; Kaczur and Cawlfield 1993). Aieta *et al.* (1986) reported that chlorine dioxide exists as a dissolved gas over the pH range from 2 to 10 and begins to disproportionate into chlorite and chlorate at pH greater

than 10 with a half life of 20 minutes to 3 hours for concentrations between 5 and 10 mg/L. Higher concentrations have shorter half lives. Residual chlorine dioxide and chlorite ion may be removed by either reduced iron (Fe^{2+}) or the sulfite ion (SO^{2-}) over a pH range of 5 to 7 (Griese, Hauser et al. 1991) (Gordon and Rosenblatt 1995). Removal of chlorate ions remains a concern since it is unaffected by either reduced iron or sulfite ions and is generally a byproduct of chlorine dioxide generation and photodecomposition (Griese, Kaczur et al. 1992).

Chlorine

Chlorine has traditionally been the disinfectant of choice for drinking water in North America, mainly because of low cost and ease of use. Typically, chlorine gas is dissolved into water then mixed into the treatment stream. Once dissolved in water chlorine gas hydrolyzes to form hypochlorous acid, chloride ions and hydrogen ions. Following hydrolysis hypochlorous acid ionizes to form hypochlorite ions and hydrogen ions in proportions dependent on solution pH. At pH greater than 4 and dilute concentrations almost all the chlorine exists as hypochlorous acid. As pH increases above 4 the percent of free chlorine present as hypochlorous acid decreases and the percent of hypochlorite ion increases (James M. Montgomery Consulting Engineers 1985). The sum of these two components is measured as free chlorine. At pH 6, 8 and 11 and 5 °C, hypochlorous acid is 98 %, 36 % and <0.1 %, respectively, of the measured free chlorine content. These proportions were calculated using an acid dissociation constant calculated from Morris's best fit function at T = 278 K (Morris 1966).

$$pK_a = \frac{3000}{T} - 10.0686 + 0.0253 \times T \qquad \textbf{Equation 7}$$

Ozone

Ozone is a highly reactive unstable gas, which is produced on site by passage of clean dry air or oxygen through an electrical discharge. As oxygen molecules pass through the electrical discharge sufficient energy, in the form of electrons, is provided to split some oxygen molecules into oxygen ions. These ions then combine with intact oxygen molecules to form ozone (Rakness, Renner et al. 1987). In aqueous solution ozone follows two reaction pathways, direct oxidation of substrate and autodecomposition (James M. Montgomery Consulting Engineers 1985). A paper by Gordon *et al.* (1993) presented the generally accepted autodecomposition mechanism suitable for the neutral to alkaline pH range. This presented mechanism is based on a numerical model simulation study by Chelkowska *et al.* (1992) of the Tomiyasu *et al.* model (1985). The autodecomposition rate of ozone increases with increases in temperature and increases in pH above 7. Conditions favorable to the direct reaction pathway are pH less than 7 and high alkalinity (James M. Montgomery Consulting Engineers 1985).

A brief description of design requirements for the four components, feed gas preparation ozone generator, contact basin and off gas destruction units, of an air fed ozone system are provided by Rakness *et al.* (1987). These authors recommended a feed gas supply unit consisting of compressors, air dryers and filters. The compressors should be capable of meeting pressures suitable for operation and desiccant dryer air purging. Dryers should be capably of meeting an operating dew point of less than -60 °C at 1 atmosphere pressure. Air filtration is required to, protect the desiccant from oil and

moisture droplets, to remove particulates which would cause desiccant plugging and to reduce maintenance on the generator dielectric. Feed gas supplied to the ozone generator must be free of oil, moisture and particulates, thus ensuring electron flow across the discharge gap is uniform and non arcing. Since ozone decomposes at high temperatures cooling is required to remove excess heat from the generator. The ozone off gas destruction unit is typically thermal or thermal/catalyst type Rakness *et al.* (1987)

Chloramines

Chloramines are formed when chlorine reacts with ammonia nitrogen to form monochloramine, dichloramine and trichloramine. James M. Montgomery Consulting Engineers (1985) presents the following brief overview of chloramine chemistry. The combination of chloramine species is dependent on, the molar ratio of chlorine to ammonia-nitrogen, temperature, pH and alkalinity. At chlorine to ammonia molar ratios up to 1:1 monochloramine and dichloramine form in proportions dependent on pH. At pH 6 dichloramine will be approximately 40 percent of chloramines present in solution at 25°C (Safe Drinking Water Committee 1980). Decreasing pH below 6 increases the proportion of dichloramine present, increasing pH above 8 results in monochloramine only. Reduction of pH to less than 3 results in predominately trichloramine. As the chlorine to ammonia molar ratio exceeds 1:1 chloramines are oxidized to form nitrous oxide and nitrogen gas.

Disinfection Byproducts and Health Affects

A consequence of using oxidizing agents to reduce the microbial threat present in water sources is the potential generation of disinfection byproducts. Many of these byproducts are considered to be potential carcinogens, mutagens, cause of damage to internal organs or a cause of neurological damage (Bull, Birnbaum et al. 1995; Tibbetts 1995). Evidence of adverse health effects resulting from chlorination disinfection byproducts stems from results of epidemiology and toxicology studies. Toxicology studies, involving a limited number of chlorinated by-products at high doses, using experimental animals most frequently show liver and kidney damage (Craun, Bull et al. 1994). Craun also reported that some epidemiology studies suggest a relationship of colon, bladder and rectal cancers to chlorinated waters following long term exposure. In 1992 the editor and editorial board of the publication from the International Life Sciences Institute conference, on The Safety of Water Disinfection concluded (Craun 1993):

- available toxicological evidence of human health risks associated with chlorine, chloramine, chlorine dioxide, and ozone disinfection is not conclusive;
- epidemiologic evidence of increased cancer risk due to consumption of chlorinated drinking water is equivocal; and
- conference findings were insufficient to alter the WHO IARC conclusions that evidence supporting carcinogenicity of chlorinated drinking water was inadequate, thus chlorinated water was not classifiable as to its carcinogenicity.

These same conclusions were supported by the USEPA in 1995 by L. S. Birnbaum of the Health Effects Research Laboratory of the USEPA who stated that the association

of chlorination and adverse health effects in the general population remain to be proven (Bull, Birnbaum et al. 1995).

Disinfection By-Products of Ozone

Singer (1993) reported two principle classes of byproducts potentially resulting from ozonation, specifically oxidation byproducts and brominated by-products. Brominated by-products result as ozone oxidizes bromide to hypobromous acid. The hypobromous acid then becomes involved with substitution reactions with natural organic matter to produce brominated by-products. Ozonation byproducts may be involved in reactions with secondary disinfectant applications, for example chloral hydrate may be formed from acetaldehyde reactions with chlorine. The acetaldehyde may be formed during a preceding ozonation stage (Singer 1993). Compounds in each class are listed in Table 3.

Table 3, Principal ozonation by-products

Oxidation By-products		Brominated by-products
Aldehydes	Acids	
Formaldehyde Acetaldehyde Glyoxal Methyl glyoxal	Oxalic acid Succinic acid Formic acid Acetic acid	Bromate Brominated acetic acids Brominated acetonitriles Bromoform Bromopicrin

Source: (Singer 1993)

Disinfection By-Products of Chlorine Dioxide

Adverse health effects relating to chlorine dioxide and the immediate inorganic byproducts chlorite and chlorate are summarized by Condie (1986) and Gates *et al.* (1995) and are shown to involve blood, thyroid and possibly reproductive and developmental neurotoxicity effects. The USEPA following a review of available toxicological data has proposed residual limits of: 1.0 mg/L with a goal of 0.08 mg/L for chlorite; and 0.8 mg/L with a goal of 0.3 mg/L for chlorine dioxide. Chlorite, the byproduct of most concern, is produced when chlorine dioxide reacts as an oxidant to receive one electron or is present as non-reacted raw material from certain chlorine dioxide generation processes (Gordon and Rosenblatt 1995). The Chemical Manufactures Association has sponsored further toxicological studies (Gates and Harrington 1995) intended to fill data gaps responsible for the USEPA setting high uncertainty factors for calculation of maximum goal levels for chlorine dioxide and chlorite.

Richardson *et al.* (1994) have identified over forty additional disinfection byproducts resulting from chlorine dioxide treatment of a raw river water source (TOC = 1.5 mg/L, 3.0 mg/L chlorine dioxide applied, 1.14 to 1.21 mg/L residual chlorine dioxide). Richardson *et al.* (1994) also reported that many of the compounds are of unknown toxicology.

Disinfection By-Products of Free Chlorine

In the aquatic environment chlorinated by-products are produced by three basic reaction mechanisms of organic precursors with hypochlorous acid (White 1992). The three mechanisms are:

- addition of chlorine atoms to a compound;
- substitution of chlorine atoms in place of an atom present in the compound; and
- oxidation.

Of these three mechanisms it is the addition and substitution reactions which are responsible for the formation of chlorinated organic compounds. Table 4 summarizes some chlorinated by-products of concern.

Disinfection By-Products of Chloramines

Monochloramine is generally reported to be an effective method for controlling trihalomethane formation and is a much weaker oxidizing agent than free chlorine (Singer 1993). Chlorinated organic material does result from treatment with monochloramine however levels formed are considerably lower then levels which would be formed by free chlorine (Singer 1993). Principle chloramination byproducts are listed in Table 5.

Table 4 Principal chlorination by-products

Class	Compounds
Trihalomethanes	Chloroform Bromodichloromethane Dibromochloromethane Bromoform
Haloacetic acids	Monochloroacetic acid Dichloroacetic acid Trichloroacetic acid Monobromoacetic acid Dibromoacetic acid
Haloacetonitriles	Trichloroacetonitrile Dichloroacetonitrile Bromochloroacetonitrile Dibromoacetonitrile
Haloketones	1,1-Dichloropropanone 1,1,1-Trichloropropanone
Others	Chloropicrin Cyanogen chloride Chloral hydrate Chlorinated furanones

Source: (Trussell 1993)

Table 5, Principal chloramination by-products

Class	Compound
Trihalomethanes	Chloroform Bromodichloromethane Bromoform
Haloacetic acids	Dichloroacetic acid Trichloroacetic acid
Haloacetonitriles	Dichloroacetonitrile Dibromoacetonitrile
Haloketones	1,1-Dichloropropanone
Others	Chloropicrin Cyanogen chloride Aldehydes

Source: (Singer 1993; Trussell 1993)

Mechanisms of Disinfection

James M. Montgomery Consulting Engineers (1985) reported two types of mechanisms as the main factors contributing to a disinfectants efficacy. The first mechanism is oxidation of cellular walls to result in cell lysis and the second mechanism is the ability to diffuse into the cell and interfere with vital cell functions. Green and Strumpf (1946) demonstrated that trace level chlorine inactivation of bacteria resulted from inhibition of a key enzyme process. Green and Strumpf (1946) concluded that chlorine penetrated the cell wall to react with the triosephosphoric dehydrogenase enzyme. Loss of this enzyme function resulted in death by preventing cellular oxidation of glucose. Further experiments on bacterial spores led Green and Strumpf (1946) to conclude that the higher spore resistance to chlorine in comparison to vegetative cells, resulted from the greater resistance of different essential enzyme systems. Fair *et al.* (1948) speculated that it is the rate of diffusion of the disinfecting agent through the cell wall that determined the rate and relative efficiency of the disinfectant. Venkobachar *et al.* (1977) studied the effect of chlorine on oxygen consumption, phosphate uptake, cell zeta potential and leakage of cell internal macromolecules of *Escherichia coli*. These researchers concluded that the following sequence of events occur; first is reaction with the cell membrane followed by adverse effects to cell vital functions and then physical, chemical and biochemical changes to the cell membrane.

Jacangelo *et al.* (1991) studied mechanisms of inactivation of *E. coli* B by monochloramine and concluded that inactivation resulted from reactions with multiple proteins or protein mediated cell processes. Additionally these authors report

monochloramine easily passes through the cell envelope without causing significant damage to the cell wall. These researchers further suggested that the typical shoulder formation of inactivation-time data results from the time required for reactions at several sensitive cell sites to occur and not due to bacterial clumping or time required for cell penetration.

Benarde *et al.* (1967) in a study of chlorine dioxide disinfection kinetics on *E. coli* demonstrated that exposure to chlorine dioxide did not result in cell wall damage sufficient to permit leakage of interior components. Benarde *et al.* (1967) concluded that chlorine dioxide inhibited cell protein synthesis and did not inhibit vital enzyme systems. Berg *et al.* (Berg, Roberts et al. 1986) studied inactivation of *E. coli* by assessing the effect of chlorine dioxide on the outer membrane permeability to macromolecules and potassium and the effect on respiration. Berg *et al.* (Berg, Roberts et al. 1986) suggested that inactivation occurred primarily as a result of lost permeability control through the outer membrane. These authors also confirmed that concurrent damage to the cell membrane was insufficient to permit leakage of cell macromolecules.

Ozone has the highest oxidation potential of commonly used water disinfectants. Katzenelson *et al.* (1974) observed that the redox potential of the test solution increased with increasing ozone concentration up to 0.20 mg/L and leveled off at higher concentrations. Katzenelson *et al.* (1974) reported two stage inactivation curves for *E. coli*, poliovirus type 1 and coliphage T2 and a general lack of dose response for ozone concentrations above 0.2 mg/L. These authors suggested an association between redox potential and ozone mode of action. Wallis *et al.* (1990) speculated (on the basis of

electron microscopy observations) that ozone inactivation of *Giardia* cysts result from damage to the cyst wall, plasma membrane and cytoplasm.

Combined and Sequential Disinfection

There is little information on protozoan cyst or oocyst inactivation resulting from different disinfectants applied in a combined or sequential manner. Sequential application of two different disinfectants is common practice in many water treatment plants, resulting from the need for an efficient on site agent and a long lasting agent to ensure a minimum residual concentration in the distribution system. Venczel *et al.* (Venczel, Arrowood et al. 1996) compared free chlorine (5 mg/L 4 hours, 22°C) to a mixed disinfectant system (5 mg/L total oxidant, 4 hours, 22°C) using bench scale batch reactor tests and reported *C. parvum* inactivation levels of 0 and 3.8 log inactivation units, respectively. The composition of the mixed oxidant solution is presumed to contain free chlorine, hydrogen peroxide, ozone and other short lived oxidants based on the equipment manufacturers technical guide. However this was not verified in the text. Kouame and Haas (1991) demonstrated a synergistic effect on inactivation of *E. coli* when free chlorine and monochloramine are both present in a continuos stirred tank reactor system at pH 8 and 20 °C. These authors noted that the synergistic effect increased with increased residence time. Although the authors recognized that the synergism mechanism was unknown, they speculated that sublethal injury caused by one disinfectant substantially enhanced sensitivity to the second disinfectant. Katz *et al.* (1994) studied the effect of combined equal doses of chlorine dioxide and chlorine on the inactivation of indicator organisms contained in activated sludge. These authors reported an improved inactivation of total

coliforms, fecal coliforms, fecal streptococci, and coliphages following combined application of 5 mg/L each for chlorine dioxide and chlorine. However Katz *et al.* (1994) did not report the expected inactivation which would result from a single application of each disinfectant. Kott *et al.* (1980) studied the effects of ozone and chlorine applied individually, sequentially and combined on the inactivation of *Salmonella typhimurium*, poliovirus type 1 and T_2 and T_3 coliphages in secondary wastewater and artificially polluted tap water. These authors reported that simultaneous application of chlorine and ozone was superior to sequential application. In these experiments the authors also reported chlorine to be more effective than ozone for inactivation of *S. typhimurium*, fecal coliforms and *Streptococcus fecalis* and the opposite for inactivation of poliovirus type 1, and T_2 and T_3 coliphages. Unfortunately test apparatus and methods are not sufficiently described in this paper to fully understand these interesting results.

3. Materials and Methods

Oxidant Stock Solutions

Ozone

Stock ozone solution for experiments was prepared by bubbling ozone gas through two 500 mL gas absorption flasks in series, each containing 400 mL of Ultra Pure deionized water (Elga® Fisher Scientific Ltd., Pittsburgh, PA) at room temperature. Ozone gas was produced by passage of extra dry oxygen through a water cooled corona discharge ozone generator (Model T-218, Welsbach Ozone Systems Corporation, Sunnydale, CA), operated at 27.6 KPa, 100 watts and an oxygen flow rate of 0.5 sLpm. Concentration of stock ozone solution typically varied from 20 to 27 mg/L at the end of the absorption period, as measured spectrophotometrically at 260 nm. Resulting dosing stock was allowed a minimum of 10 min. to stabilize before use in experiments. Ozone stock was prepared as required and not stored.

Chlorine

Stock chlorine solution was prepared by dilution of sodium hypochlorite solution (6 % available chlorine, BDH Inc. Poole England) with oxidant demand free water to provide a concentration of 300 ± 10 mg/L. Chlorine stock was diluted within two hours of requirement.

Monochloramine

Stock monochloramine solution was prepared two hours in advance of experiments by mixing equal volumes of 100 mg/L as Nitrogen of ammonium chloride (Analar grade, BDH, Poole, UK) and 300 mg/L as free chlorine of sodium hypochlorite solution. This mixture provided a 3:1 W/W (Cl_2:N) ratio and yielded a final monochloramine concentration of 150 ± 10 mg/L, after 15 min. reaction time, (Haas, Hornberger et al. 1993). Sodium hypochlorite solution was diluted with pH 8 demand free phosphate buffered water to ensure rapid chloramine formation rate when mixed with ammonium chloride solution (Safe Drinking Water Committee 1980).

Chlorine dioxide

Chlorine dioxide stock solution was generated by passage of a humidified three to five percent chlorine gas in a nitrogen carrier through a chlorine dioxide generator (CDG by Advanced Systems Technologies). The resultant chlorine dioxide gas was absorbed into 500 mL of high purity deionized water to yield a concentration of approximately 3000 mg/L of chlorine dioxide. with a chlorine content less than 3 % by weight as determined by amperometric titration method (APHA AWWA WEF 1992). Dosing stock used for experiments was obtained by diluting the generated stock with oxidant demand free water to a level of .

Oxidant Demand Free Water and Phosphate Buffers

Oxidant demand free water was made oxidant demand free by passing an ozone/oxygen gas mixture through a glass sparger into four litres of high quality deionized

water (Elga® Fisher Scientific Ltd., Pittsburgh, PA). Following forty minutes of aeration, using oxygen gas containing approximately 5% (w/w) ozone, was a forty minute soak period and finally the water was boiled rapidly for a minimum of 15 min. Prepared water was allowed to cool to room temperature and transferred into ozone demand free glass containers for storage.

0.05 M phosphate pH 6 buffer solution at was prepared by mixing 0.869 g/L of di-sodium hydrogen orthophosphate (Analar grade, BDH, Poole, UK) and 6.818 g/L of potassium di-hydrogen orthophosphate (Analar grade, BDH, Poole, UK) in high quality deionized water. 0.05 M phosphate pH 8 buffer solution was prepared by mixing 7.098 g/L of di-sodium hydrogen orthophosphate and 0.426 g/L of reagent grade potassium di-hydrogen orthophosphate in high quality deionized water. 0.05 M phosphate pH 11 buffer solution was prepared by mixing 7.098 g/L of di-sodium hydrogen orthophosphate and adding potassium hydroxide (Analar grade, BDH, Poole, UK) as required in high quality deionized water. Buffered solutions were made oxidant demand free following the procedure specified for oxidant demand free water.

Demand Free Glassware and Utensils

The use of oxidant demand free glassware, laboratory waters and utensils in experiments ensured oxidant demand resulted only from the cyst suspension. Sequential disinfection experiments utilized glassware and utensils made oxidant demand free to the first disinfectant. All demand free waters were ozone demand free. All glassware and utensils were protected from airborne contaminants by storage in oxidant demand free containers capped with aluminum foil.

Ozone Demand Free Glassware and Utensils

Ozone demand free glassware and utensils were prepared by immersion in ozone solution (minimum 10 mg/L ozone), capped using aluminum foil then allowed to soak for a minimum of six hours at room temperature (Haas, Hornberger et al. 1993). On completion of the soak period, glassware and utensils were drained and dried thoroughly at a minimum of 70 °C. Ozone solution was prepared by aeration of four litres of high quality deionized water using a glass sparger and an ozone/oxygen mixture for a minimum of forty minutes. Ozone/oxygen gas mixture was prepared by methods described previously.

Chlorine Dioxide Demand Free Glassware and Utensils

Chlorine dioxide demand free glassware and utensils were prepared by immersion in chlorine dioxide solution (200 to 400 mg/L), capped using aluminum foil and soaked in the dark for a minimum of 24 hours at room temperature (APHA AWWA WEF 1992). On completion of the soak period, glassware and utensils were rinsed three times with oxidant demand free water then dried at a minimum of 70 °C. Chlorine dioxide solution was prepared by dilution of chlorine dioxide stock with Elga Ultra Pure water..

Chlorine and Monochloramine Demand Free Glassware

Chlorine demand free glassware and utensils were prepared by immersion in chlorine solution at10 to 50 mg/L (APHA AWWA WEF 1992), capped using aluminum foil and allowed to soak for a minimum of twelve hours at room temperature. On completion of the soak period, glassware and tips were rinsed three times with oxidant

demand free water then dried thoroughly at a minimum of 70 °C. Chlorine solution was prepared by dilution of 6 % sodium hypochlorite solution with Elga Ultra Pure water. Glassware and utensils used for monochloramine experiments were made chlorine demand.

Residual Oxidant Measurement

Chlorine and Monochloramine

Free chlorine and monochloramine concentrations were determined using DPD indicator reagents for free and total chlorine (PrairieChem catalog number 14070-99). Each pillow was dissolved in twenty five mL of test solution and percent absorption recorded at 515 nm. A standard curve was prepared using serial dilutions of a standard potassium permanganate (Analar grade, BDH, Poole, UK) and Elga Ultra pure water solution covering a chlorine equivalent range of 0.05 to 4.0 mg/L (APHA AWWA WEF 1992). Absorption at 515 nm was converted to mg/L as chlorine for both free and combined chlorine using the following equation:

$$-0.03272 + 3.67614 \times A \qquad \textbf{Equation 8}$$

where: A = absorbtion at 515 nm.

This equation was determined using procedures specified by Standard Methods (APHA AWWA WEF 1992). Initial and final Reactor concentrations were measured by removing twenty five mL for either free chlorine or total chlorine DPD analysis. Chlorine reactions were quenched using either 0.100 mL of a 0.1 N sodium thiosulfate solution or

with an equal volume dose of 100 mg/L ammonium chloride. The ammonium chloride solution was used when monochloramine followed as a the secondary disinfectant.

Ozone

Ozone concentrations were measured directly by ultraviolet spectrophotometry at 260 nm and converted to mass units using a molar absorption coefficient of 3,300 $M^{-1} cm^{-1}$ (Hart, Sehested et al. 1983; Langlais, Reckhow et al. 1991).

The ozone dose was applied as a single aliquot once the dosing stock had decayed to the required concentration. Dosing stock concentration was measured twice immediately before applying the dose to the reactor and twice following dose application. Ozone residual in the reactor was monitored continuously using the UV/VIS spectrophotometer at 260 nm at a flow rate of 11 mL/min through a 0.5 mL volume 1 cm light path length flow cell. On completion of the designated contact time residual ozone was quenched using .050 mL of 0.1 molar sodium formate solution. Sodium formate rapidly quenched the oxidant without resulting in either an interfering absorbance at 260 nm or impacting the secondary disinfectant.

Chlorine Dioxide

Chlorine Dioxide concentrations were measured spectrophotometrically in a 1 cm cell at 360 nm and converted to mass units using a molar absorption coefficient of 1250 $M^{-1} cm^{-1}$ (Gordon, Cooper et al. 1987).

Volume of dosing stock required for 1.0 mg/L was applied in one or two aliquots using a demand free pipette. Residual disinfectant concentration in the reactor was

continuously monitored using the UV/VIS spectrophotometer at 360 nm at a flow rate of 11 mL/min through a 0.5 mL volume one cm light path length flow cell. A molar absorption coefficient of 1250/M/cm (Gordon, Cooper et al. 1987) for absorbtion of chlorine dioxide at 360 nm results in a conversion factor of 53.96 given a molecular weight of 67.45 g. Reactors were dosed at 1.0 mg/L following cyst addition. Reactions were quenched using 0.041 mL of 0.10 molar sodium thiosulfate. An excess quench was required to end reactions rapidly for accurate disinfectant contact time control.

Parasites

The parasite selected for this study is a strain of *Giardia muris*, originally isolated from a Golden hamster by Roberts Thompson *et al.* (Roberts-Thomson, Stevens et al. 1976). This strain is maintained in a P–2 SPF facility by passage through either SCID or CD1 mice (Belosevic and Faubert 1983).

Cyst Collection and Isolation

G. muris cysts that were used in experiments were obtained from fecal pellets produced by female CD-1 mice during 4 to 10 days following infection by gavage of 40,000 to 50,000 cysts. To obtain fecal pellets, mice were placed daily in a false bottom cage for 2 to 3 h and resulting pellets were collected for cyst isolation. Isolation of cysts from the pellets was completed within 4 h of collection. The method of isolation used was the sucrose gradient technique described by Roberts-Thompson *et al.* (Roberts-Thomson, Stevens et al. 1976) and modified by Belosevic and Faubert (Belosevic and Faubert 1983) with a substitution of deionized Milli-Q® (OM-140; Millipore Corp. Bedford, Mass.)

water for saline solution. A total of twenty mice were infected weekly in two groups, 10 on Tuesday and 10 on Thursday to provide a combined total of 9×10^6 cysts for each collection day. Cyst collection from all mice commenced on the Monday following infection.

The isolation procedure followed is summarized as follows: Fecal pellets were softened in approximately 30 mL of Milli-Q® water for 30 min., before being emulsified using 5 mm diameter wooden applicator sticks. Approximately 2 mL volume of the fecal slurry was layered onto 2.5 mL of a 1 M sucrose solution (1.12 s.g. at 4 °C) in 75 × 15 mm glass tubes and centrifuged at 1200 rpm for 15 min. (Jouan 4.11 swinging bucket centrifuge, 175 mm radius). The cyst containing band at the water - sucrose interface was removed by Pasteur pipette, and placed into a 15 mL conical centrifuge tube (up to a maximum of 5 mL) for washing. The remaining tube volume was filled with deionized Milli-Q® water, gently mixed by inversion and centrifuged at 1400 rpm for 10 min. (Jouan 4.11 swinging bucket centrifuge, 184 mm radius). The supernatant was removed to less than 0.5 mL, the cyst pellet resuspended and the wash step repeated once. The supernatant from the second wash was removed and the cyst pellet resuspended in 1.0 mL of Milli-Q® water for storage at 4 °C.

Prior to use in experiments cyst stock from several days was combined and washed one to two times at 1200 rpm for 10 min. (Jouan 4.11 swinging bucket centrifuge, 184 mm radius) in oxidant demand free water to ensure absorbance at 260 nm was ≤ 0.020/cm for a cyst concentration of 10,000 cysts/mL. Following the final wash the supernatant was

removed and the pellet resuspended in an appropriate volume of oxidant demand free water.

Cysts were used within four days from isolation. Cyst stock older than four days was discarded.

Cyst Enumeration

Cyst concentration was determined from the average of replicate counts of two aliquots from each of two diluted samples, using a Neuhauser hemacytometer. Four squares from each aliquot were counted and averaged to provide an estimate of the cyst density per square. The diluted cyst concentration in cysts per mL was calculated by multiplying average cysts per square by 10,000. The concentration of cysts in the stock solution was determined by multiplying the diluted concentration by the appropriate dilution factor.

Excystation Procedure

The excystation procedure described by Schaefer *et al.* (Schaefer, Rice et al. 1984; 1990), with modifications described below, was used to measure cyst viability. There are three steps in Schaefer's excystation procedure, specifically induction, wash and incubation. The induction step proceeded in the following manner: the cyst sample was transferred by glass Pasteur pipette into a 15 mL conical centrifuge tube, the original container was rinsed twice using 0.3 mL Elga Ultra pure water each time and the rinse water was added to the transferred cyst solution. The remaining volume was filled with equal volumes of modified reducing solution followed by freshly mixed bicarbonate

solution. The filled tube was capped securely, gently mixed by inversion three times and placed in a dark incubation chamber for 30 min. at 37.5 C. All solutions used for the induction step were pre-warmed to 37 °C

The wash step proceeded as follows:

1. immediately following induction the solution tube was centrifuged at 1200 rpm for 10 min. at 4°C;
2. the supernatant was then carefully aspirated to leave 0.5 mL;
3. the cyst pellet was gently re-suspended in the remaining supernatant;
4. a ten milliliter volume of 4°C modified Trypsin Tyrode's solution was added and mixed gently using a Pasteur pipette;
5. the suspension was centrifuged at 1200 rpm for 10 min. at 4°C.;
6. the supernatant was carefully aspirated to leave approximately 0.75 milliliters; and
7. the pellet was gently resuspended in the remaining supernatant using a glass Pasteur pipette.

The third step was completed by incubating the resuspended solution in the dark at 37.5 C for 30 min.

Modified reducing solution was prepared daily in a 40 mL volume as follows: 21.6 mL modified Hank's solution (0.400 g/L potassium chloride (Analar grade, BDH, Poole, UK), 0.060 g/L potassium di-hydrogen orthophosphate (Analar grade, BDH, Poole, UK), 8.000 g/L sodium chloride (Analar grade, BDH, Poole, UK), 0.120 g/L di-sodium hydrogen orthophosphate, 0.350 g/L sodium hydrogen carbonate, 1.000 g/L D-Glucose

(ACS Fisher Scientific, Edmonton Alberta), in Elga Ultra Pure water); 6.80 mL glutathione solution (0.24 g glutathione (G-6013 Sigma Chemical Co. St. Louis, Mo.) in 8.0 mL of modified Hank's) and 11.6 mL of L-cysteine solution (0.42 g L-cysteine (C8277 Sigma Chemical Co. St. Louis, Mo.) in 15.0 mL of modified Hank's solution). Bicarbonate solution was prepared by adding 10 mL Elga Ultra Pure water at 37.5°C to 0.84 g of sodium bicarbonate (Fisher Scientific code S233-500). Modified Trypsin Tyrode's solution was prepared as follows: 40.0 mL modified Tyrode's solution (0.20 g/L potassium chloride (Analar grade, BDH, Poole, UK), 0.06 g/L potassium di-hydrogen orthophosphate, 8.00 g/L sodium hydrogen carbonate (Analar grade, BDH, Poole, UK), 0.05 g/L di-sodium hydrogen orthophosphate, 1.00 g/L sodium hydrogen carbonate, 1.00 g/L D-Glucose plus 0.20 g of either Trypsin Ty II crude from Porcine Pancreas (T8128 Sigma Chemical Co. St. Louis, Mo.) or Trypsin Ty II-S (T7409 Sigma Chemical Co. St. Louis, Mo.). Use of Trypsin Ty II crude from Porcine Pancreas required vigorous mixing, centrifugation at 1960 rpm for 10 min. in a 50 mL conical tube followed by vacuum filtration through a 0.45 μm polycarbonate filter. Trypsin Type II-S dissolved completely and was used without the centrifugation and filtration steps, no difference in excystation rates was observed with the Trypsin substitution. Modified Hank's and modified Tyrode's solutions were prepared monthly and stored in the dark at 5°C, all other solutions were prepared daily.

Disinfection Procedures

All experiments were conducted in bench scale batch reactors using laboratory waters at 5 ± 1°C and in batch reactors. The disinfection procedure followed is based on

work by Labatiuk *et al.* (1991). Four different disinfectants (ozone, chlorine, monochloramine or chlorine dioxide) were used in either single or sequential experiments. Target disinfectant dosage and contact times for each pH are summarized in Table 6. Typical experimental setup is shown by Figure 1

Demand free batch reactors for all experiments consisted of 250 mL Erlenmeyer flask containing 200 mL of oxidant demand free water. Reactors were protected from stray light by an aluminum foil cover and maintained at 5 ± 1°C in an ice water bath. Mixing was achieved by using Teflon coated magnetic stir bars. Stir bar rpm adjusted to maintain a vortex depth of 1 to 2 cm to ensure rapid mixing of added solutions and to maintain cysts in suspension. Prior to addition of disinfectant, each reactor was seeded with approximately 2×10^6 cysts. After allowing sufficient time for cysts to mix thoroughly the disinfectant dose was injected below the water surface using a mass calibrated pipette. At completion of the contact period, an appropriate volume of quenching agent was added to the reactor. Initial and final temperatures and residual disinfectant concentrations were recorded by methods described previously.

Sequential disinfectant experiments continued with the addition of a second disinfectant dose applied between 15 and 30 minutes after the first disinfectant was stopped, with the exception of chlorine followed by monochloramine experiments. The second disinfectant dosage was performed in a manner similar to first dose with the following exceptions:

- In chlorine followed by monochloramine experiments, the chlorine dose was stopped by an equal volume of ammonium chloride solution which also

resulted in formation of monochloramine. To permit the use of equal volumes, chlorine dose stock was set at 300 mg/L and ammonium chloride dose stock was set at 100 mg/L as nitrogen. This method provided a simple procedure for ensuring a 3:1 Cl_2:N_2 weight ratio was maintained. Monochloramine was quenched using a surplus of sodium thiosulfate at the end of the contact period.

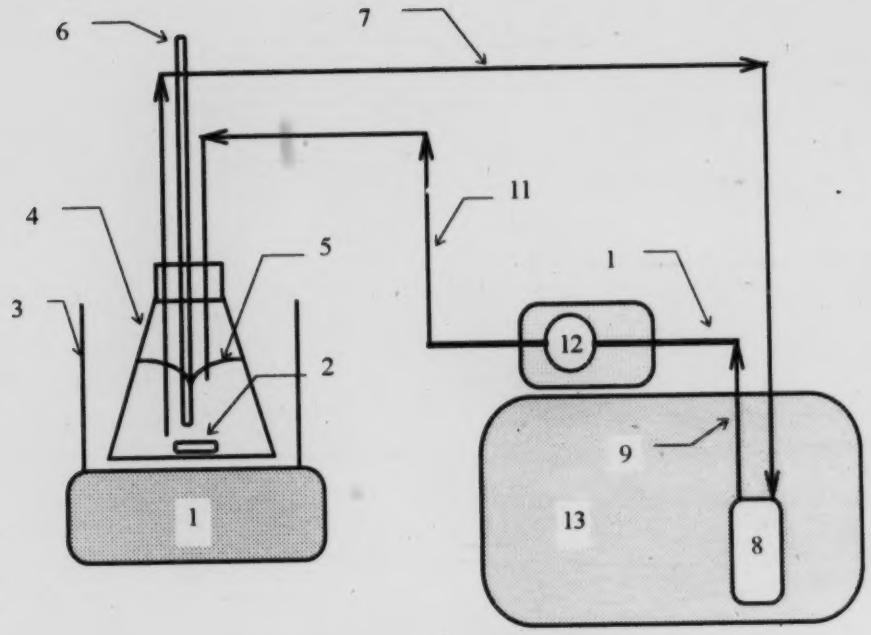
- In the ozone followed by either chlorine or monochloramine experiments, ozone was quenched using surplus sodium formate, which had no impact on subsequent application of either chlorine or monochloramine.
- In chlorine dioxide followed by either chlorine or pre-formed monochloramine experiments, chlorine dioxide was quenched with surplus sodium thiosulfate as in single dose experiments. The chlorine or pre-formed monochloramine dose was increased by a factor of 1.2 to compensate for this surplus reducing agent. This factor was empirically determined to result in an initial secondary dose concentration of 2 mg/L as chlorine.

Initial and final temperatures and disinfectant residual levels were recorded for each disinfection step. Following either primary quenching for single dose experiments or secondary quenching for sequential experiments, a sufficient volume of 1.0 % vol/vol Tween 20 was added to each reactor to result in a final reactor concentration of 0.01% vol/vol Tween 20. Reactor contents were then transferred to a 175 mL plastic conical centrifuge tube and stored at 4°C until start of the excystation procedure.

Table 6 Target disinfectant dosage

Disinfectant	Primary Dose Applied. mg/L	Secondary Dose Applied. mg/L	pH	Contact Time
Chlorine	2.0	2.0	6	30 min.
		2.4*	8	60 min.
			11	60 min.
Monochloramine	2.0	2.0	8	150 min.
		2.4*	11	5 min.
Chlorine Dioxide	1.0	1.0	6	10 min.
			8	5 min.
			11	5 sec.
Ozone	0.2	0.2	6	60 sec.
			8	17 sec.
			11	5 sec.

Note: * applied concentration when following chlorine dioxide dose.



1	magnetic stir plate	8	0.5 mL 1 cm light path length flow cell HP model 5061-3398
2	Teflon coated magnetic stir bar	9	flow cell exit to peristalic pump line, 20 cm length
3	ice bath	10	Peristalic pump tube, Tygon 2.79 mm dia. 27 cm length
4	250 mL Erlenmeyer flask, covered with aluminum foil	11	HP teflon return line to reactor, 19 cm length
5	typical vortex formation, 1 to 2 cm	12	HP peristalic pump model 89052B
6	mercury thermometer (ACS Fisher Scientific, Edmonton Alberta)	13	HP 8452A UV/VIS Diode Array spectrophotometer, with HP Vectra ES/12 computer
7	HP teflon inflow line to flow cell, 40 cm length		

Figure 1 Experimental apparatus setup for continuous monitoring of ozone and chlorine dioxide levels.

4. Results

Prior to performing sequential disinfection experiments, the expected inactivation resulting from individual doses was determined experimentally. A dose concentration typical of a water treatment plant (James M. Montgomery Consulting Engineers 1985) was selected for each disinfectant except ozone. The ozone dose was determined from disinfection efficacy and sensitivity of ozone residual measurement equipment. For each disinfectant, a series of randomized individual log inactivation tests were conducted at varying CT values with constant concentration. Results of these tests are contained in appendix A. Ct values for each disinfectant were then selected from this data base for use in sequential experiments. The expected log inactivation for the individual CT doses applied in sequential tests are summarized in Table 1. Table 2 summarizes Ct values and results from sequential experiments.

Viability of cysts was determined by in-vitro excystation for both disinfection tests and control tests. Excystation levels of cysts from control reactors were consistently above 90%. Excystation results from control reactors are included with single dose experimental results in appendix C.

Table 7 Summary of single dose test results.

Test	pH	n	Ct	Average dose		Inactivation ratio Log units	
			(mg/L)min.	mg/L	s.e.	ave.	s.e.
Cl_2	6	2	57	1.91	0.06	-0.73	0.00
Cl_2	8	4	119	1.99	0.03	-0.58	0.08
Cl_2	11	2	116	1.94	0.06	-0.04	0.01
NH_2Cl	8	2	303	2.02	0.01	-0.50	0.13
NH_2Cl	11	3	9	1.81	0.05	-0.71	0.12
ClO_2	6	3	10	0.98	0.02	-0.72	0.09
ClO_2	8	2	5.7	1.15	0.15	-0.74	0.06
ClO_2	11	4	0.08	1.13	0.06	-0.74	0.07
O_3	6	3	0.17	0.17	0.01	-0.49	0.06
O_3	8	3	0.05	0.19	0.01	-0.76	0.11
O_3	11	2	0.01	0.13	0.01	-0.42	0.06

notes:

1. All tests conducted in batch reactors using phosphate buffered solution at 5° C ± 1° C.
2. Inactivation ratio measured by *in vitro* excystation.
3. n = sample size.
4. s.e. = standard error.
5. Disinfectant dose is an arithmetic average of initial and residual values

Free Chlorine

Examination of the data in Table 1 clearly demonstrates the decreasing efficacy of free chlorine as pH was increased from 6 to 8 to 11. At pH 8 the Ct value is almost twice the value required at pH 6 and at pH 11 a Ct value comparable to the value at pH 8 was ineffective at inactivation of cysts.

Monochloramine

Examination of the data in Table 1 clearly demonstrates a dramatic increase in monochloramine efficacy as pH was increased from 8 to 11. At pH 8 a Ct value of 303 resulted in a -0.50 log inactivation ratio while at pH 11 the required Ct decreased to 9 for a log inactivation ratio of -0.71.

Chlorine Dioxide

Examination of the data in Table 1 clearly demonstrates the increasing efficacy of chlorine dioxide as pH was increased from 6 to 8 to 11. At pH 8 the Ct value is half of the value required at pH 6, however at pH 11 there was a dramatic decrease in Ct required for a log inactivation ratio of -0.74. Replication of inactivation experiments was dependent on repeating the dose pattern as described by the spectrophotometer absorbance verses time profile. Typical spectrophotometer scan profiles at 360 nm, for chlorine dioxide dosage at pH 6, 8 and 11 are shown in appendix B.

Ozone

Examination of the data in Table 1 demonstrates a dramatic increase in ozone effectiveness from pH 6 to pH 8. At pH 6 a Ct value of 0.17 resulted in a log inactivation level of -0.49 while a Ct of 0.05 at pH 8 resulted in a log inactivation level of -0.76. At pH 11 ozone effectiveness increased slightly over that at pH 8 where a Ct value of 0.01 resulted in a log inactivation level of -0.42. Replication of inactivation experiments was dependent on repeating the dose pattern as described by the spectrophotometer absorbance verses time profile. Replication of ozone dosage at pH 11 was difficult due to the increased decay rate of ozone in solution. Typical spectrophotometer scan profiles at 260 nm, for ozone dosage at pH 6, 8 and 11 are shown in appendix B.

Sequential Experiments

Sequential experiments were conducted by applying previously evaluated single experiment dosages in a sequential manner. The secondary dose was applied within thirty minutes following quench of the primary disinfectant dose. Table 2 summarizes excystation levels resulting from sequential experiments. Synergistic effects, as determined by the difference between measured sequential inactivation and the sum of expected inactivations resulting from single doses, are summarized in Table 3 and illustrated in Figure 1 through Figure 5. A strong synergistic effect is demonstrated when the effect is greater than would be expected by strict summation of individual dose inactivation levels plus the sum of standard errors from single and sequential inactivations. Strong synergistic effects at pH 6 and pH 8 was demonstrated for all combinations tested, excluding the chlorine dioxide/monochloramine combination. The chlorine

dioxide/monochloramine combination produced a slight synergistic effect at pH 8. At pH 11 only the ozone/monochloramine combination produced a synergistic effect. Generally synergistic effects were higher at lower pH than at higher pH levels. The only sequential combination which produced a synergistic effect at pH 11 was the ozone/monochloramine combination.

Table 8 Summary of sequential test results

Test			First disinfectant			Second disinfectant			Log inactivation ratio	
Sequence	pH	n	Ct (mg/L)min.	ave. C. mg/L	s.e.	Ct (mg/L)min.	ave. C. mg/L	s.e.	ave.	s.e.
ClO_2- Cl_2	6	3	10.10	1.01	0.02	53.1	1.77	0.05	-2.31	0.15
ClO_2- Cl_2	8	3	4.95	0.99	0.02	106	1.77	0.03	-2.32	0.29
ClO_2- NH_2Cl	8	3	5.20	1.04	0.02	268.5	1.79	0.03	-1.74	0.27
ClO_2- NH_2Cl	11	3	0.08	1.02	0.01	8.4	1.68	0.14	-1.53	0.28
O_3 - Cl_2	6	2	0.20	0.17	0.01	52.5	1.75	0.01	-2.30	0.08
O_3 - Cl_2	8	2	0.06	0.19	0.01	115	1.92	0.02	-2.21	0.21
O_3 - Cl_2	11	2	0.01	0.13	0.03	110.4	1.84	0.10	-0.40	0.11
O_3 - NH_2Cl	8	2	0.06	0.20	0.01	291	1.94	0.01	-2.10	0.01
O_3 - NH_2Cl	11	2	0.01	0.14	0.01	9.2	1.84	0.01	-1.76	0.08
Cl_2- NH_2Cl	8	2	116	1.94	0.03	295	1.97	0.01	-2.43	0.14
Cl_2- NH_2Cl	11	2	105	1.76	0.11	9.0	1.81	0.07	-0.72	0.26

notes:

1. All tests conducted in batch reactors using phosphate buffered solution at 5° C ± 1° C.
2. Inactivation ratio measured by *in vitro* excystation.
3. n = sample size.
4. s.e. = standard error.
5. Disinfectant dose is an arithmetic average of initial and residual values

Table 9. Synergistic effects

	pH	Measured sequential inactivation		Sum of Expected inactivations		Synergistic Effect $x_s - x_i - \sum s.e.$
		Average Log units x_s	Standard error s.e.	Average Log units x_i	Standard error s.e.	Log units
Chlorine - Monochloramine	8	-2.43	0.14	-1.08	0.21	-1
Chlorine - Monochloramine	11	-0.72	0.26	-0.75	0.13	0.00
Chlorine Dioxide - Chlorine	6	-2.31	0.15	-1.45	0.09	-0.62
Chlorine Dioxide - Chlorine	8	-2.32	0.29	-1.31	0.15	-0.57
Chlorine Dioxide - Monochloramine	8	-1.74	0.27	-1.24	0.20	-0.03
Chlorine Dioxide - Monochloramine	11	-1.53	0.28	-1.45	0.19	-0.00
Ozone - Chlorine	6	-2.30	0.08	-1.22	0.06	-0.94
Ozone - Chlorine	8	-2.21	0.21	-1.34	0.19	-0.47
Ozone - Chlorine	11	-0.40	0.11	-0.46	0.07	0.00
Ozone - Monochloramine	8	-2.10	0.01	-1.26	0.24	-0.59
Ozone - Monochloramine	11	-1.76	0.08	-1.13	0.17	-0.38

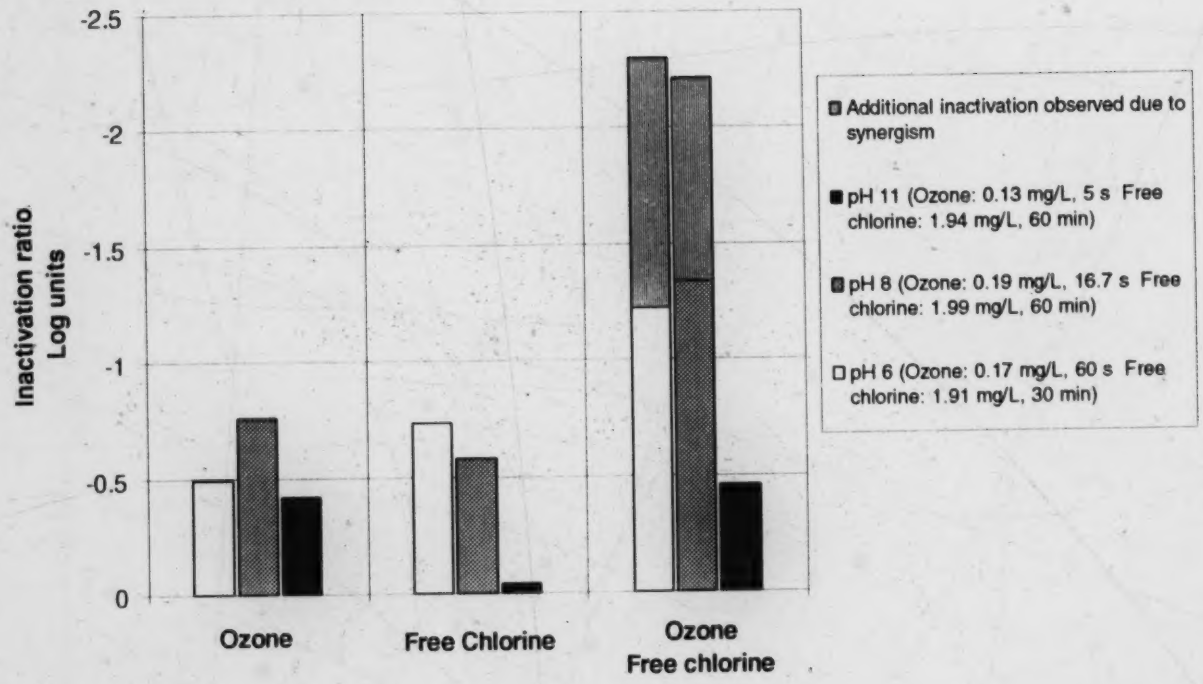


Figure 2. Inactivation of *G. muris* cysts by ozone followed by free chlorine at pH 6, 8 and 11.

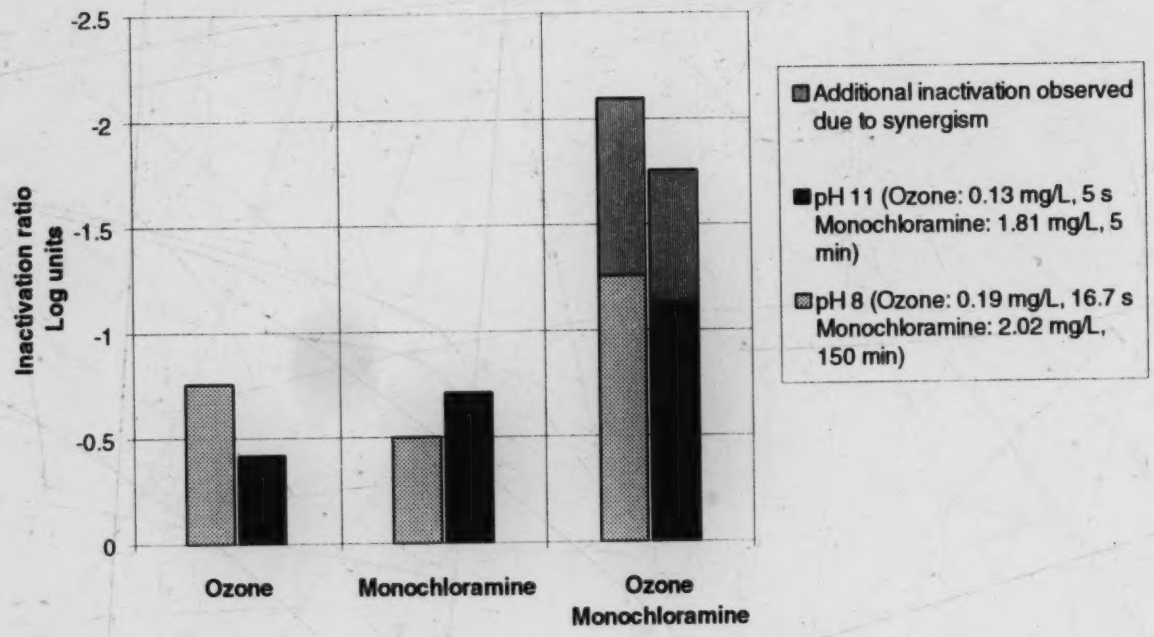


Figure 3. Inactivation of *G. muris* cysts by ozone followed by monochloramine at pH 8 and 11.

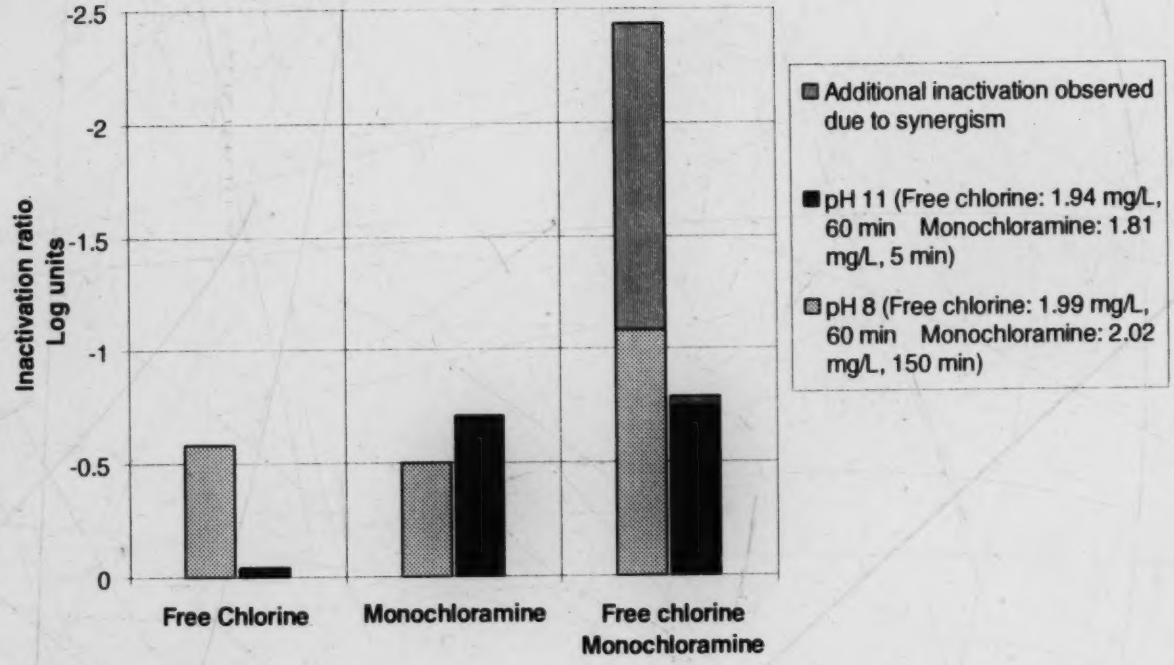


Figure 4. Inactivation of *G. muris* cysts by free chlorine followed by monochloramine at pH 8 and 11.

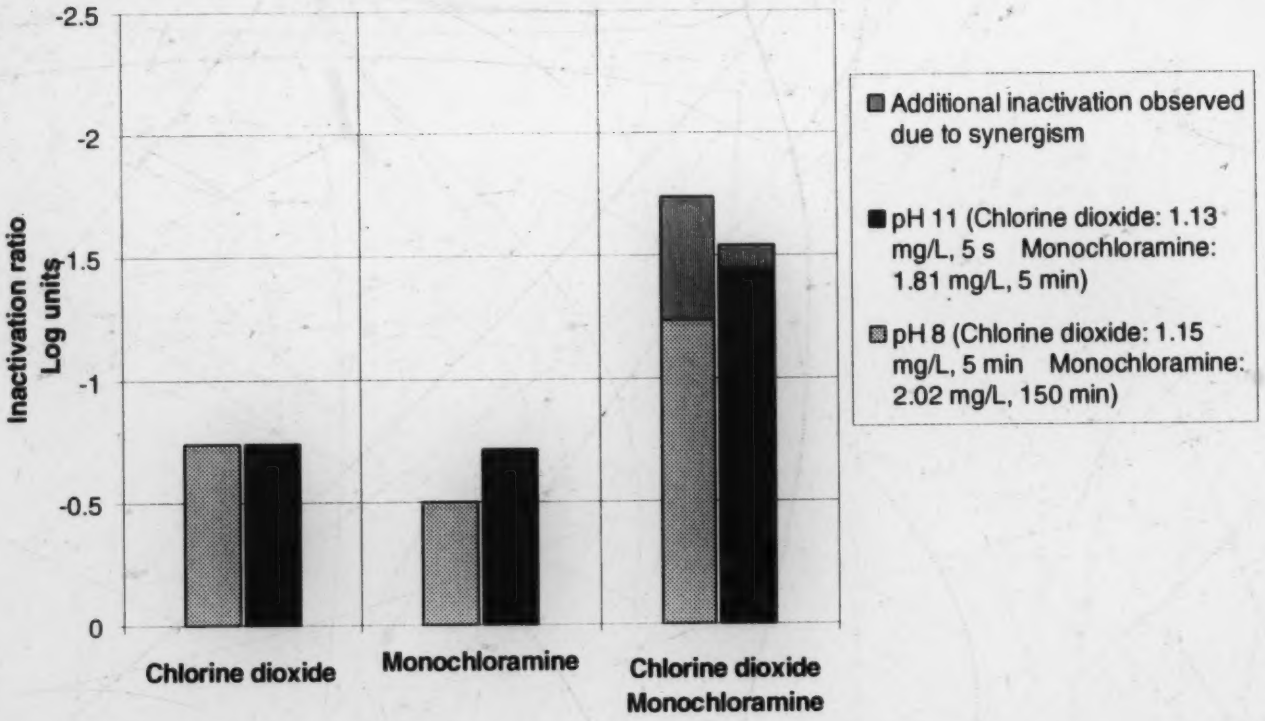


Figure 5. Inactivation of *G. muris* cysts by chlorine dioxide followed by monochloramine at pH 8 and 11.

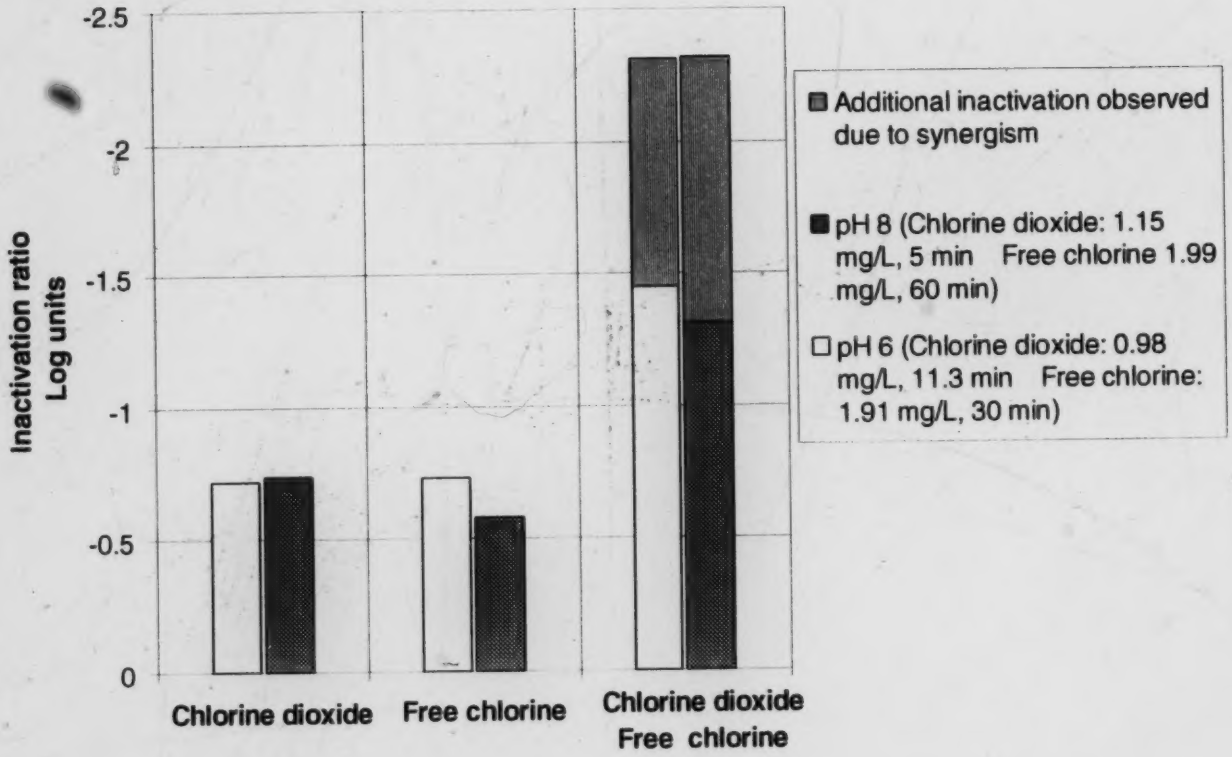


Figure 6. Inactivation of *G. muris* cysts by chlorine dioxide followed by free chlorine at pH 6 and 8.

5. Discussion

Single Inactivation Test Results

A comparison of Ct values presented by the USEPA to Ct values resulting from this study is summarized in Table 10. The USEPA Ct values listed in the table have been linearly extrapolated between tabulated values and adjusted to account for safety factors applied by the USEPA. Ct values for monochloramine inactivation at pH 8 in this study are similar to the USEPA values for the same inactivation level, specifically 303 for this study versus 365 from the USEPA. Comparable USEPA Ct values for *G. muris* cyst inactivation by chlorine dioxide are 4.3 with no change for pH differences. Results from this study show higher Ct values of 10 at pH 6 and 5.7 at pH 8. These differences may be attributed to the limited data used by the USEPA to determine their recommended values, differences in cyst sources and experimental protocols. USEPA chlorine dioxide Ct values were based on pH 7 test results and not adjusted for pH effects on efficacy, however the USEPA recognizes that chlorine efficacy increases with increasing pH. Ozone Ct value of 0.17 at pH 6 for -.5 log inactivation from this study compares with 0.16 presented by the USEPA. Ct values at for similar inactivation levels at higher pH levels in this study decreased as pH increased. Ct values for chlorine, in this study, are approximately twice as high as those presented for *Giardia lamblia* by the USEPA. Possible reasons for this may be morphological differences between the two species, difference between *in vitro* excystation of *G. muris* versus animal infectivity of *G. lamblia* as well as differences in excystation procedures used by the various researchers. These results support reports claiming *G. muris* to be more free chlorine resistant than *G. lamblia* and further supports

the use of *G. muris* as conservative model for *G. lamblia* in disinfection studies. Free chlorine was the only disinfectant evaluated which did not show increasing efficacy with increasing pH. This decreasing efficacy of free chlorine is a consequence of the increasing proportion of hypochlorite ion molar concentration to hypochlorous acid molar concentration and the *G. muris* cyst resistance to hypochlorite ion. If the CT values are compared on the basis of hypochlorous acid concentration and time then the Ct value would be approximately two thirds less at pH 8 than at pH 6 for the same free chlorine concentration. Consequently it is likely that the efficacy of hypochlorous acid may not be affected by changes to pH, however further experimentation is required for confirmation of this hypothesis.

Table 10. Comparison of Ct values to USEPA published values

Test	pH	Inactivation ratio Log units ave.	Ct (mg/L)min. This study	Ct (mg/L)min. EPA [3,4,6]
Cl_2	6	-0.73	57	28
Cl_2	8	-0.58	119	48
Cl_2	11	-0.04	116	N/A
NH_2Cl	8	-0.50	303	365
NH_2Cl	11	-0.71	9	N/A
ClO_2	6	-0.72	10	4.3
ClO_2	8	-0.74	5.7	4.3
ClO_2	11	-0.74	0.08	N/A
O_3	6	-0.49	0.17	.16
O_3	8	-0.76	0.05	.16
O_3	11	-0.42	0.01	N/A

notes:

1. All tests conducted in batch reactors using phosphate buffered solution at 5° C ± 1° C.
2. Inactivation ratio measured by *in vitro* excystation.
3. EPA Ct for ozone divided by 2 (EPA safety factor).
4. EPA Ct for chlorine dioxide is divided by 1.5 (EPA safety factor).
5. Disinfectant dose is an arithmetic average of initial and residual values
6. EPA Ct values taken from "Guidance manual for compliance with the Surface Water Treatment Requirements for Public Water Systems. Criteria and Standards Div., USEPA Ofce. Of Drinking Water. Washington D. C. (Nov.1986).

Sequential Inactivation Test Results

Sequential Experimental Results

The synergistic effect resulting from sequential application of two disinfectants was determined by comparing measured inactivations from sequential tests to the sum of expected log inactivations from respective single dose tests. The significance of the synergistic effects was measured by a paired t test of the difference in means. A total of five pairs on disinfectants at various pH levels were tested and compared. Table 9 presents a summary of log inactivation results and the measured synergistic effects. A synergistic effect is demonstrated when the difference in averages, between measured sequential inactivation levels and expected inactivation levels, is greater than the sum of standard errors.

Synergistic effects are demonstrated for the following pairs of disinfectants:

- chlorine followed by monochloramine at pH 8, illustrated by Figure 4
- chlorine dioxide followed by chlorine at pH 6, illustrated by Figure 6;
- chlorine dioxide followed by chlorine at pH 8, illustrated by Figure 6;
- ozone followed by chlorine at pH 6, illustrated by Figure 2;
- ozone followed by chlorine at pH 8, illustrated by Figure 2;
- ozone followed by monochloramine at pH 8, illustrated by Figure 3; and
- ozone followed by monochloramine at pH 11, illustrated by Figure 3.

An absence of a synergistic effect was demonstrated for the following pairs:

- chlorine followed by monochloramine at pH 11, illustrated by Figure 4;
- chlorine dioxide followed by monochloramine at pH 8, illustrated by Figure 5;
- chlorine dioxide followed by monochloramine at pH 11, illustrated by Figure 5; and
- ozone followed by free chlorine at pH 11, illustrated by Figure 2.

Examination of the synergistic effects at the various pH levels reveals a trend of decreasing synergistic effect with increasing pH. The sole exception was chlorine dioxide followed by free chlorine where net synergistic effects were -.62 and -.57 inactivation log units at pH 6 and pH 8 respectively. This result is interesting since it indicates that this synergistic effect is constant and independent of pH over a pH range of 6 to 8. Further interpretation of this result is complicated by differences in concentration of hypochlorous acid resulting impact on diffusion through the cyst and cellular membranes. Further experiments are required to eliminate effects of this variable on the resulting synergism. The absence of a synergistic effect at pH 11 supports the theory that cyst wall permeability increases with increasing pH. Therefore it follows that at pH 11 diffusion through the cyst wall and membrane is at a maximum due to high pH and the diffusion rate of the second disinfectant was not appreciably enhanced. Additionally an absence of synergism occurs at pH 11 when free chlorine is one of the sequential disinfectants due to the absence of hypochlorous acid. The absence of a synergism effect with the chlorine dioxide followed by monochloramine experiments, at both pH 8 and pH 11, supports current mechanism

theories. Specifically the absence of a clear synergistic effect indicates that chlorine dioxide passes through the cyst wall and cell membranes without causing sufficient or appropriate membrane damage to facilitate passage of the subsequent monochloramine dose. This result also supports the theory of different inactivation target sites for different disinfectants. If following penetration of the cyst wall and membranes chlorine dioxide and monochloramine then react at different cellular sites to cause death then no synergistic effect is possible. In contrast the ozone followed by monochloramine combinations showed clear synergistic effects at pH 8 and pH 11, supporting the theory that ozone causes sufficient damage to the cyst wall and membranes to facilitate penetration of the subsequent monochloramine or free chlorine dose. The increased efficacy of ozone at higher pH levels may be consequence of improved cyst wall and membrane permeability and the presence of ozone decomposition radicals.

6. Conclusions

1. This study successful demonstrated the presence of a synergistic effect, on the inactivation of *G. muris* cysts, from sequential application of carefully selected disinfectants at an appropriate pH.
2. The magnitude of the synergistic effect decreases with increasing pH. It was also observed that efficacy of the disinfectants increased with increasing pH, free chlorine excepted.
3. The synergistic effect may be the result of an increased penetration rate of the secondary disinfectant, as a consequence of damage to cyst wall or membranes caused by the primary disinfectant.

4. Results from this study clearly demonstrate the potential exists for water treatment plants to achieve required levels of pathogen inactivation and reduced disinfection by-products levels through planed sequential application of multiple disinfectants.

7. Recommendations for Future Work

Although this study has successfully demonstrated a synergistic effect on *G. muris* cyst inactivation, considerable work remains to be conducted before guidelines may be prepared for use by the water treatment industry. The following factors remain to be addressed:

- demonstration of a synergistic effect using other microorganism types;
- magnitude of temperature effects;
- development of kinetic models.
- the use of high inactivation methods such as animal infectivity or flow cytometry is recommended to more measure the magnitude of synergistic effects at higher Ct values.
- optimize concentrations and contact time for various combinations of disinfectants; and
- the impact of natural waters on the synergistic effect.

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Appendix A

Table A.11 Ozone Disinfection Test Results

Date	Exp. #	Comments	Test pH	Contact Time minutes	pH 6		pH 8		pH 11	
					Log Inactivation	Meas. Ave. mg/L	Log Inactivation	Meas. Ave. mg/L	Log Inactivation	Meas. Ave. mg/L
5/14/96	64		11	0.08					-0.47	0.14
7/17/96	136		11	0.08					-0.36	0.11
5/11/96	61		11	0.17					-0.81	0.08
4/4/96	25		8	0.25			-0.54	0.19		
7/17/96	135		8	0.25			-0.87	0.20		
4/23/96	41		6	0.33	-0.19	0.15				
5/11/96	62		8	0.33			-0.87	0.19		
5/11/96	63		11	0.42					-1.26	0.12
4/3/96	22		8	0.50			-0.75	0.18		
4/4/96	26		8	0.50			-0.59	0.17		
4/4/96	27		8	0.75			-0.95	0.19		
4/23/96	42		6	0.83	-0.37	0.17				
7/17/96	134		6	1.00	-0.53	0.18				
3/4/96	23		8	1.00			-1.74	0.18		
5/1/96	45		6	1.17	-0.56	0.17				
4/23/96	43		6	1.50	-0.62	0.17				
5/1/96	47		11	1.50					-1.25	0.07
5/1/96	46		6	3.00	-1.43	0.17				
		Summary	sample size			6		7		5
			average, mg/L			0.17		0.19		0.10
			standard error			0.004		0.004		0.013

Table A.12 Chlorine Dioxide Disinfection Test Results

Date	Exp. #	Comments	Test pH	Contact Time minutes	pH 6		pH 8		pH 11	
					Log Inactivation	Meas. Ave. mg/L	Log Inactivation	Meas. Ave. mg/L	Log Inactivation	Meas. Ave. mg/L
5/4/96	53		11	0.08					-0.55	1.05
7/5/96	125		11	0.08					-0.88	1.06
5/4/96	52		11	0.17					-0.90	0.99
5/4/96	54		11	0.42					-1.50	0.99
7/4/96	122	new cuvette flow tube	11	0.08					-0.81	1.11
7/4/96	121	old cuvette flow tube	11	0.08					-0.70	1.30
3/31/96	10		8	1.00			-0.09	0.98		
3/31/96	11		8	2.00			-0.16	0.97		
3/31/96	12		8	3.00			-0.24	0.92		
4/2/96	15		8	3.00			-0.32	1.02		
4/16/96	33		6	4.00	-0.12	0.91				
6/27/96	112		8	5.00			-0.80	1.00		
7/4/96	123		8	5.00			-0.67	1.30		
4/2/96	16		8	6.00			-0.63	1.12		
4/16/96	34		6	8.00	-0.26	0.92				
4/2/96	17		8	9.00			-0.90	1.10		
6/28/96	116		6	10.00	-0.82	1.01				
4/18/96	39		8	10.00			-0.81	1.02		
4/16/96	35		6	12.00	-0.55	0.95				
4/18/96	38		6	12.00	-0.79	0.97				
4/18/96	37		6	24.00	-1.29	0.99				
		Summary	sample size			6		9		5
			average, mg/L			0.96		1.05		1.08
			standard error			0.02		0.04		0.05

Table A.13 Chlorine

Date	Exp. #	Test	Test pH	Contact Time minutes	pH 6		pH 8		pH 11	
					Log Inactivation	Meas. Ave. mg/L	Log Inactivation	Meas. Ave. mg/L	Log Inactivation	Meas. Ave. mg/L
6/21/96	109	in ClO2 byproducts	6	30	-0.73	1.85				
5/31/96	91		6	30	-0.73	1.97				
3/24/96	1		8	30			-0.16	2.04		
4/14/96	31		6	40	-0.76	1.77				
5/2/96	51		11	60					-0.03	1.88
4/11/96	29		6	60	-1.34	1.85				
3/24/96	2		8	60			-0.37	2.01		
5/23/96	79		8	60			-0.67	2.03		
7/27/96	111		8	60			-0.53	1.92		
6/21/96	108	in ClO2 byproducts	8	60			-0.74	1.98		
3/24/96	3		8	90			-0.85	1.95		
5/2/96	50		11	180					-0.05	1.99
		Summary	sample size			4		6		2
			average, mg/L			1.86		1.99		1.94
			standard error			0.04		0.02		0.06

Table A.14 Monochloramine Disinfection Test Results

Date	Exp. #	Comments	Test pH	Contact Time minutes	pH 8		pH 11	
					Log Inactivation	Meas. Ave. mg/L	Log Inactivation	Meas. Ave. mg/L
5/23/96	78		11	5			-0.50	1.87
6/8/96	106		11	5			-0.90	1.84
7/5/96	126		11	5			-0.73	1.72
5/17/96	74		11	30			-1.14	1.97
5/17/96	75		11	50			-1.00	2.09
5/15/96	71		11	70			-1.18	1.99
5/17/96	76		11	70			-1.15	2.01
5/15/96	72	Pre-chlorinated	11	70			-1.15	2.01
5/9/96	56		11	120			-0.99	1.95
5/9/96	58		11	180			-1.33	2.02
3/26/96	5		8	90	-0.10	2.00		
3/26/96	6		8	120	-0.27	2.00		
3/26/96	7		8	150	-0.37	2.01		
7/12/96	132	Pre-chlorinated	8	150	-0.63	2.03		
3/26/96	8		8	180	-0.57	1.99		
		Summary	sample size			5		10
			average, mg/L			2.01		1.95
			standard error			0.01		0.03

Appendix B

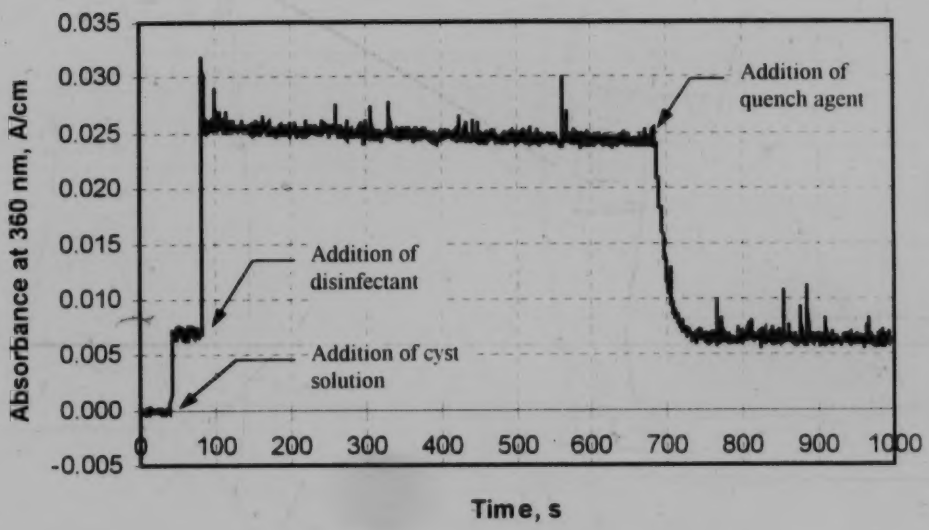

Figure B.7: Typical spectrophotometer scan for chlorine dioxide at pH 6.

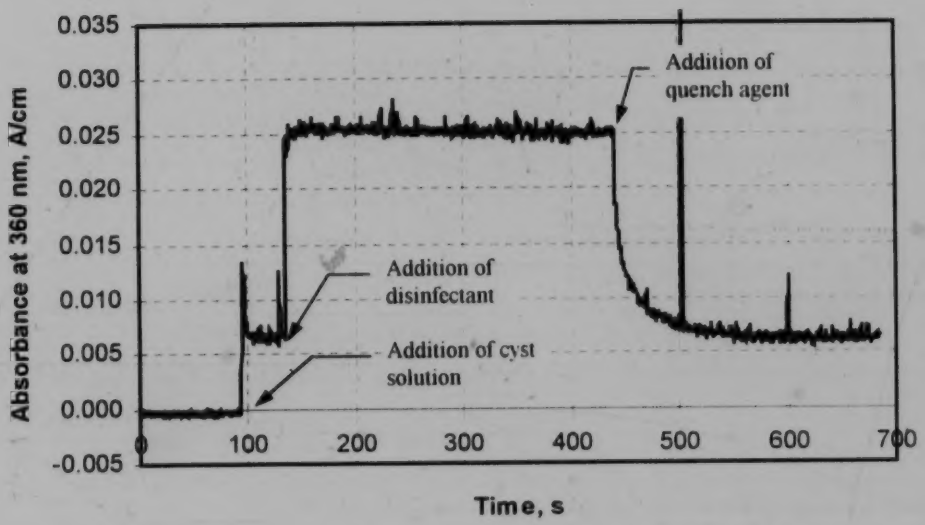

Figure B.8: Typical spectrophotometer scan for chlorine dioxide at pH 8.

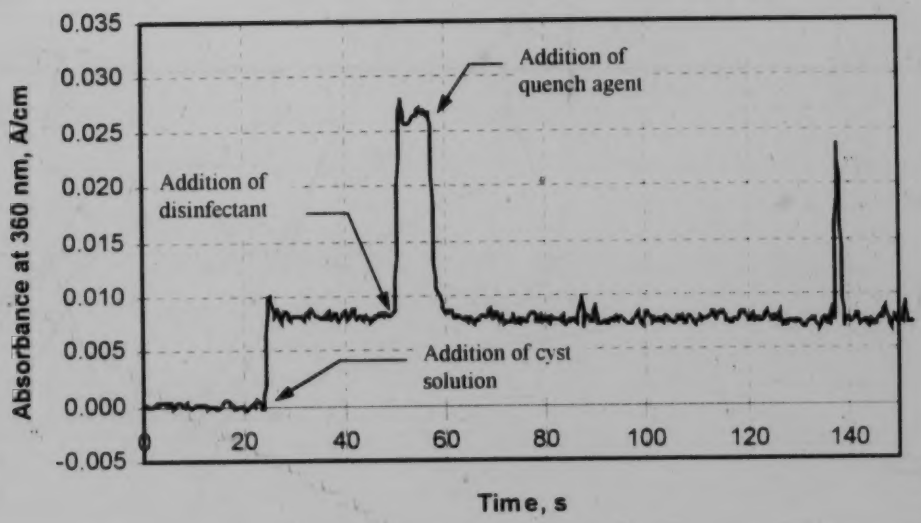

Figure B.9: Typical spectrophotometer scan for chlorine dioxide at pH 11.

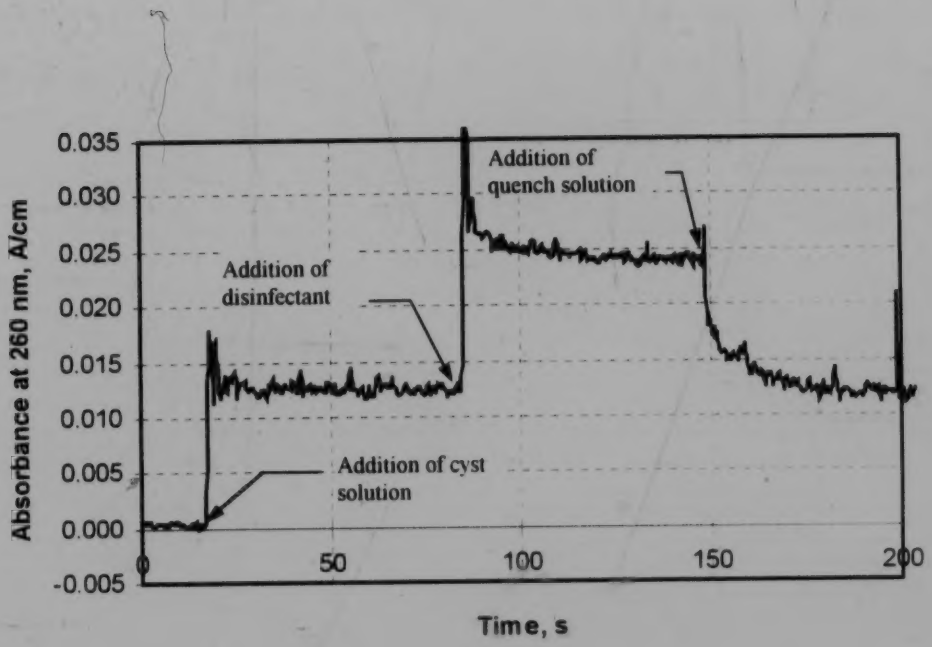

Figure B.10: Typical spectrophotometer scan for ozone at pH 6.

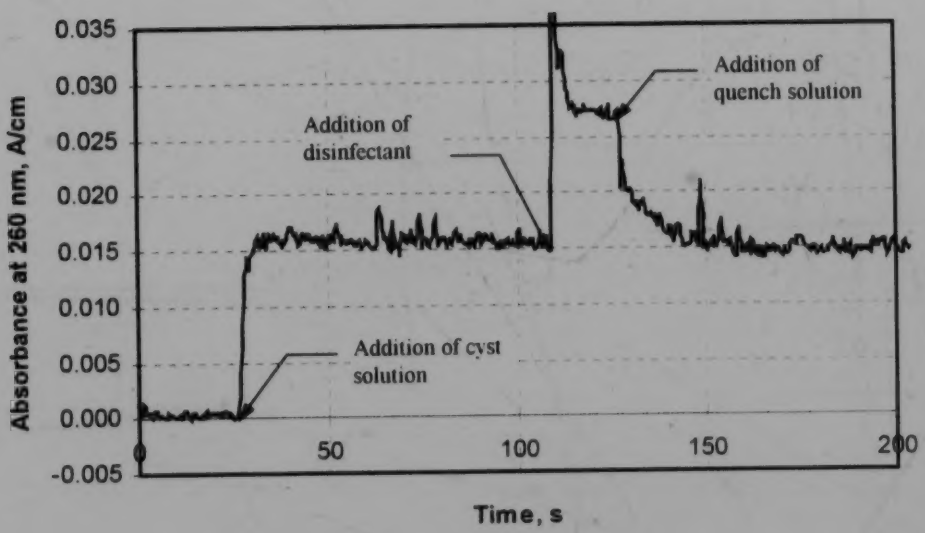

Figure B.11: Typical spectrophotometer scan for ozone at pH 8.

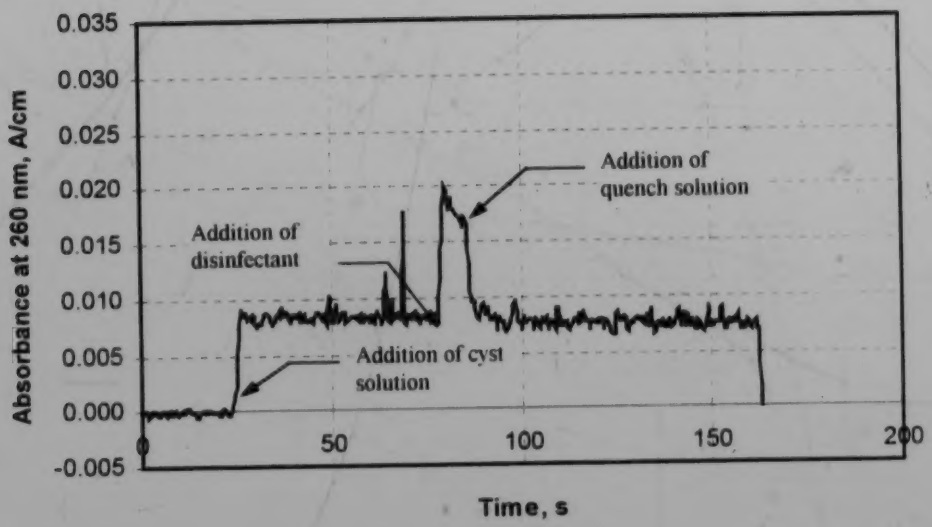

Figure B.12: Typical spectrophotometer scan for ozone at pH 11.

Appendix C

Table C1. Ozone Dose Data

Date	Exp. #	Test	%A_{260} O_3 stock				Ave. O_3 mg/L	Mass added gm	Vol. Added mL
			Before Dosing		After Dosing				
4/3/96	21	O_3	1.61	1.61	1.5	1.55	24.36	1.65	1.654
4/3/96	22	O_3	1.655	1.67	1.649	1.601	25.54	1.65	1.654
3/4/96	23	O_3	1.658	1.64	1.51	1.588	24.85	1.65	1.654
4/4/96	25	O_3	1.675	1.67	1.677	1.644	25.89	1.685	1.689
4/4/96	26	O_3	1.7	1.64	1.658	1.631	25.75	1.685	1.689
4/4/96	27	O_3	1.681	1.66	1.626	1.601	25.53	1.685	1.689
4/23/96	41	O_3	1.724	1.71	1.688	1.647	26.31	1.651	1.655
4/23/96	42	O_3	1.739	1.74	1.728	1.724	26.94	1.651	1.655
4/23/96	43	O_3	1.712	1.7	1.687	1.682	26.33	1.651	1.655
5/1/96	45	O_3	1.701	1.69	1.679	1.65	26.1	1.67	1.674
5/1/96	46	O_3	1.714	1.69	1.674	1.662	26.18	1.67	1.674
5/1/96	47	O_3	1.713	1.71	1.695	1.671	26.38	1.67	1.674
5/11/96	61	O_3	1.711	1.7	1.663	1.674	26.21	1.69	1.694
5/11/96	62	O_3	1.709	1.69	1.663	1.65	26.07	1.69	1.694
5/11/96	63	O_3	1.756	1.74	1.683	1.694	26.68	1.69	1.694
5/14/96	64	O_3	1.65	1.63	1.609	1.612	25.27	1.77	1.774
7/17/96	134	O_3	1.352	1.36	1.297	1.302	20.65	2.133	2.138
7/17/96	135	O_3	1.349	1.35	1.319	1.308	20.69	2.133	2.138
7/17/96	136	O_3	1.327	1.33	1.329	1.357	20.77	2.133	2.138
5/14/96	65	O_3 - Cl_2	1.643	1.64	1.59	1.58	25.08	1.77	1.774
5/14/96	67	O_3 - Cl_2	1.642	1.62	1.594	1.601	25.1	1.77	1.774
5/14/96	68	O_3 - Cl_2	1.618	1.6	1.623	1.575	24.93	1.77	1.774
6/2/96	100	O_3 - Cl_2	1.557	1.55	1.549	1.558	24.13	1.813	1.817
6/2/96	101	O_3 - Cl_2	1.586	1.57	1.548	1.504	24.12	1.813	1.817
6/2/96	102	O_3 - Cl_2	1.591	1.57	1.547	1.583	24.44	1.813	1.817
5/24/96	83	O_3 - NH_2Cl	1.594	1.61	1.549	1.565	24.53	1.71	1.714
5/24/96	84	O_3 - NH_2Cl	1.594	1.6	1.576	1.576	24.65	1.71	1.714
6/8/96	104	O_3 - NH_2Cl	1.526	1.54	1.522	1.503	23.64	1.81	1.814
6/8/96	105	O_3 - NH_2Cl	1.572	1.59	1.568	1.583	24.53	1.81	1.814

Table C2 Sequential Dose Experimental Results

Date	Exp #	Test	Comments	Test pH	pH	First disinfectant										
					meas	Dose Stock mg/L	Vol added mL	Applied Conc mg/L	Meas Initial mg/L	Meas Final mg/L	Meas Ave mg/L	Ratio Init /App	Contact Time		Temp Start °C	Temp End °C	Temp Ave °C
5/14/96	65	O_3 - Cl_2		11	10.93	25.1	1.77	0.22	0.15	0.15	0.15	0.68	5	sec	5.4	5.4	5.4
5/14/96	67	O_3 - Cl_2		8	7.98	25.1	1.77	0.22	0.2	0.19	0.2	0.91	15	sec	5.8	5.8	5.8
5/14/96	68	O_3 - Cl_2		6	6.02	24.9	1.77	0.22	0.2	0.18	0.19	0.91	60	sec			
6/2/96	100	O_3 - Cl_2		6	5.99	24.1	1.82	0.22	0.22	0.17	0.2	1.01	60	sec	5.5	6	5.75
6/2/96	101	O_3 - Cl_2		8	7.99	24.1	1.82	0.22	0.19	0.18	0.19	0.88	15	sec	5.1	5.1	5.1
6/2/96	102	O_3 - Cl_2		11	10.96	24.4	1.82	0.22	0.13	0.09	0.11	0.59	5	sec	5.3	5.3	5.3
6/21/96	108	Cl_2	in ClO2 byproducts	8	7.99	183	1.09	0.99	0	0	0	0	0	min	4.5	4.5	4.5
6/21/96	109	Cl_2	in ClO2 byproducts	6	6.00	183	1.09	0.99	0	0	0	0	0	min	5.8	5.8	5.8
5/9/96	57	NH_2Cl	pre-chlorination	11	10.93								0	min	4.4	4.8	4.6
5/15/96	72	NH_2Cl	pre-chlorination	11	10.96	298	1.34	1.98				0	0	min	5.6	5.6	5.6
7/12/96	132	NH_2Cl	pre-chlorination	8	7.99	304	1.32	1.98	0	0	0	0	0	min			
5/24/96	81	Cl_2 - NH_2Cl		8	8.00	303	1.33	2	1.97	1.95	1.96	0.98	60	min	5.5	5.5	5.5
5/24/96	82	Cl_2 - NH_2Cl		11	10.97	303	1.33	2	1.88	1.85	1.87	0.94	60	min	4.8	5.2	5
6/1/96	95	Cl_2 - NH_2Cl		8	7.98	300	1.33	1.98	1.94	1.88	1.91	0.98	60	min	4.5	4.5	4.5
6/1/96	96	Cl_2 - NH_2Cl		11	10.96	300	1.33	1.98	1.74	1.54	1.64	0.88	60	min	4	5.7	4.85
5/28/96	88	ClO_2 - Cl_2		8	7.98	91.7	2.21	1	1	1	1	1	300	sec	5	5.7	5.35
5/28/96	89	ClO_2 - Cl_2		6	5.99	91.7	2.21	1	0.99	0.98	0.99	0.99	10	min	4.8	4.8	4.8
5/31/96	92	ClO_2 - Cl_2	High ClO2 inactivation	8	7.98	124	1.61	0.99	0.95	0.92	0.94	0.96	300	sec	5.6	6.3	5.95
5/31/96	93	ClO_2 - Cl_2		6	6.00	124	1.61	0.99	1	0.95	0.98	1.01	10	min	5.5	6.4	5.95
6/28/96	114	ClO_2 - Cl_2		8	8.00	246	0.81	0.99	1.03	1.01	1.02	1.04	300	sec	3.5	4.8	4.15
6/28/96	115	ClO_2 - Cl_2		6	5.99	246	0.81	0.99	1.06	1.03	1.05	1.07	10	min	4.5	5.3	4.9
5/28/96	86	ClO_2 - NH_2Cl		11	10.97	91.7	2.21	1	1	1	1	1	5	sec	5.8	5.8	5.8
5/28/96	87	ClO_2 - NH_2Cl		8	7.98	91.7	2.21	1	1.02	1	1.01	1.02	300	sec	5.5	6.2	5.85
6/1/96	97	ClO_2 - NH_2Cl	High ClO2 inactivation	11	10.96	124	1.61	0.99	1.03	1.03	1.03	1.04	5	sec	4.6	4.6	4.6
6/1/96	98	ClO_2 - NH_2Cl		8	7.98	124	1.61	0.99	1.07	1.02	1.05	1.08	300	sec	4.5	6.3	5.4
7/5/96	127	ClO_2 - NH_2Cl		11	11.04	237.4	0.84	0.99	1.02	1.02	1.02	1.03	5	sec	5	5	5
7/5/96	128	ClO_2 - NH_2Cl		8	8.01	237.4	0.84	0.99	1.08	1.06	1.07	1.09	300	sec	5	6	5.5
5/24/96	83	O_3 - NH_2Cl		8	8.00	24.5	1.71	0.21	0.19	0.19	0.19	0.91	20	sec	4.6	5	4.8
5/24/96	84	O_3 - NH_2Cl		11	10.97	24.7	1.71	0.21	0.2	0.18	0.19	0.96	5	sec	6	6	6
6/8/96	104	O_3 - NH_2Cl		8	8.00	23.6	1.81	0.21	0.2	0.2	0.2	0.95	20	sec	5.4	5.4	5.4
6/8/96	105	O_3 - NH_2Cl		11	10.95	24.5	1.81	0.22	15	15	15	68.1	5	sec	4.8	4.8	4.8

Table C2 Sequential Dose Experimental Results

Date	Exp #	Second disinfectant								Temperature, °C			Control	Test		Counts		
		Dose Stock mg/L	Vol added mL	Applied Conc mg/L	Meas Initial mg/L	Meas Final mg/L	Meas Ave mg/L	Ratio Init / App	Contact Time	Start	End	Ave	pH	Excystation ratio	Excystation ratio	Log Inactivation ratio	PET	ECW	IC
5/14/96	65	319	1 254	1 98	1 95	1 93	1 94	0 98	60 sec	5	5 3	5 2	8	0 93	0 288	-0 51	78	13	225
5/14/96	67	319	1 254	1 98	1 97	1 9	1 94	0 99	60 sec	5 2	5 5	5 4	8	0 93	0 009	-2	1	5	636
5/14/96	68	319	1 254	1 98	1 79	1 66	1 73	0 9	60 sec	5 6	5	5 3	8	0 93	0 004	>2 37	1	1	503
6/2/96	100	322	1 24	1 98	1 9	1 62	1 76	0 96	30 sec	4 5	4 4	4 5	8	0 96	0 006	-2 22	5	0	865
6/2/96	101	322	1 24	1 98	1 9	1 9	1 9	0 96	60 sec	4 4	4	4 2	8	0 96	0 004	-2 42	0	3	813
6/2/96	102	322	1 24	1 98	1 74	1 74	1 74	0 88	60 sec	4 7	4 8	4 8	8	0 96	0 508	-0 28	112	52	159
6/21/96	108	310	1 68	2 57	1 99	1 96	1 98	0 77	60 min	4 5	4 5	4 5	11	0 84	0 154	-0 74	64	4	373
6/21/96	109	310	1 61	2 47	2	1 69	1 85	0 81	30 min	5 8	5 8	5 8	11	0 84	0 155	-0 73	64	10	403
5/9/96	57	150 3	3 67	2 7	2 42	3 16	2 79	0 9	120 min				10 9	0 96	0 013	-1 87	3	4	528
5/15/96	72	100	1 342	0 67	2 01	2 01	2 01	3 02	70 mn				11	0 94	0 066	-1 15	22	12	479
7/12/96	132	100	1 316	0 65	2 03	2 03	2 03	3 11	150 mn	5 9	4 5	5 2	6	0 97	0 225	-0 63	37	20	196
5/24/96	81	100	1 333	0 66	1 94	1 97	1 96		150 mn	4 8	5 2	5	8	0 94	0 005	-2 29	3	0	626
5/24/96	82	100	1 333	0 66	1 74	1 74	1 74		5 mn	5	5	5	8	0 94	0 099	-0 98	16	15	283
6/1/96	95	100	1 33	0 66	2 01	1 94	1 98		150 mn	4 8	4 8	4 8	8	0 93	0 003	-2 57	2	0	793
6/1/96	96	100	1 33	0 66	1 88	1 88	1 88		5 mn	5 4	5 4	5 4	8	0 93	0 327	-0 45	52	30	169
5/28/96	88	300 5	1 64	2 44	1 86	1 78	1 82	0 76	50 min	5 8	4 8	5 3	6	0 95	0 011	-1 96	7	0	658
5/28/96	89	300 5	1 64	2 44	2 03	1 6	1 82	0 83	30 mn	5 8	4 5	5 2	6	0 95	0 006	-2 22	4	0	688
5/31/96	92	315	1 27	1 98	1 64	1 82	1 73	0 83	60 min	6	5	5 5	8	0 97	0 001	-2 9	1	0	820
5/31/96	93	315	1 27	1 98	1 75	1 58	1 67	0 88	30 min	5	4	4 5	8	0 97	0 002	-2 61	1	1	840
6/28/96	114	304	1 579	2 38	1 76	1 76	1 76	0 74	60 min	4 5	4 5	4 5	8	0 98	0 008	-2 11	5	1	776
6/28/96	115	304	1 579	2 38	1 93	1 72	1 83	0 81	30 min	5 4	4 7	5 1	8	0 98	0 008	-2 11	5	1	787
5/28/96	86	158	3 05	2 37	1 66	1 57	1 62	0 7	5 sec	5 2	5 6	5 4	6	0 95	0 097	-0 99	48	1	457
5/28/96	87	158	3 05	2 37	1 9	1 77	1 84	0 8	150 mn	5	5 6	5 3	6	0 95	0 061	-1 19	29	0	445
6/1/96	97	153	2 614	1 97	1 48	1 48	1 48	0 75	5 sec	4 2	4 2	4 2	8	0 93	0 010	-1 95	8	0	767
6/1/96	98	153	2 614	1 97	1 81	1 63	1 72	0 92	150 min	5 3	4 7	5	8	0 93	0 009	-2 03	5	1	692
7/5/96	127	167	2 757	2 27	1 88	1 99	1 94	0 83	5 sec	4 6	4 2	4 4	11	0 94	0 021	-1 65	11	2	598
7/5/96	128	167	2 607	2 14	1 87	1 74	1 8	0 87	150 min	5	5	5	11	0 94	0 010	-1 99	6	0	615
5/24/96	83	151	2 649	1 97	1 92	1 95	1 94	0 97	150 sec	6	6 3	6 2	8	0 94	0 007	-2 1	3	2	663
5/24/96	84	151	2 649	1 97	1 85	1 85	1 85	0 94	5 sec	5 2	5 2	5 2	8	0 94	0 020	-1 68	11	1	598
6/8/96	104	134	2 985	1 97	1 92	1 94	1 93	0 98	150 min	4 8	3 8	4 3	8	0 89	0 007	-2 09	4	2	821
6/8/96	105	134	2 985	1 97	1 78	1 86	1 82	0 9	5 min	5	5	5	8	0 89	0 013	-1 83	10	1	819

Table C3. Single Dose Experimental Results

Date	Exp #	Lab Book page	Test	Comments	Test pH	pH	First disinfectant										Control			Test		Counts		
						mean	Dose Stock mg/L	Vol added mL	Applied Conc mg/L	Mean Initial mg/L	Mean Final mg/L	Mean Ave mg/L	Ratio Init /App	Contact Time	Temp Start °C	Temp End °C	Temp Ave °C	pH	Excystation ratio	Excystation ratio	Log Inactivation ratio	PET	ECW	IC
3/24/96	1	C117	Cl_2		8	8.00	280	1.43	1.98	2.07	2	2.04	1.01	30 min	5	5	5	8	0.95	0.652	-0.16	156	26	97
3/24/96	2	C117	Cl_2		8	8.00	280	1.43	1.98	2.07	1.94	2.01	1.04	60 min	4.6	4.2	4.4	8	0.95	0.403	-0.37	96	6	151
3/24/96	3	C117	Cl_2		8	8.00	280	1.43	1.98	1.92	1.98	1.95	0.97	90 min	4.5	5.6	5.05	8	0.95	0.134	-0.85	48	0	311
4/11/96	29	C156	Cl_2		6	5.98	289	1.38	1.98	2.03	1.66	1.85	1.03	60 min	3.5	3.4	3.45	6	0.95	0.044	-1.34	17	2	416
4/14/96	30	C162	Cl_2		6	5.96	280	1.43	1.98	1.94	1.66	1.8	0.98	20 min	4	3.6	3.8	6	0.95	0.641	-0.17	132	20	85
4/14/96	31	C162	Cl_2		6	5.96	280	1.43	1.98	1.86	1.68	1.77	0.94	40 min	4.2	3	3.6	6	0.95	0.164	-0.76	49	7	285
5/2/96	50	D17	Cl_2		11	11.00	270	1.48	1.98	1.99	1.98	1.99	1	180 min	4.8	5.4	5.1	11	0.96	0.859	-0.05	65	45	18
5/2/96	51	D17	Cl_2		11	11.00	270	1.48	1.98	2	1.75	1.88	1.01	60 min	4.3	5.4	4.85	11	0.96	0.886	-0.03	60	64	16
5/23/96	79	D59	Cl_2		8	8.00	300	1.33	1.98	2.08	1.98	2.03	1.05	60 min	4	5.2	4.6	11	0.93	0.201	-0.67	49	1	199
5/31/96	91	D83	Cl_2		6	6.00	315	1.27	1.98	2	1.94	1.97	1.01	30 min	4.9	5.6	5.25	8	0.97	0.182	-0.73	42	6	216
6/27/96	111	D150	Cl_2		8	8.00	301	1.33	1.98	2.03	1.81	1.92	1.02	60 min	5.4	3.2	4.3	8	0.94	0.280	-0.53	72	9	298
7/12/96	130	D173	Cl_2		6	5.98	304	1.32	1.98	1.7	1.89	1.8	0.86	30 min	5.6	6.2	5.9	6	0.97	0.239	-0.61	54	3	181
3/31/96	10	C129	ClO_2		8	8.00	56.7	3.53	0.98	0.98	0.97	0.98	1	60 sec	5.5	5.5	5.5	8	0.96	0.772	-0.09	161	59	65
3/31/96	11	C129	ClO_2		8	8.00	56.7	3.53	0.98	0.98	0.95	0.97	1	120 sec	4.6	4.6	4.6	8	0.96	0.661	-0.16	196	15	108
3/31/96	12	C129	ClO_2		8	8.00	56.7	3.53	0.98	0.96	0.09	0.53	0.98	180 sec	5.6	5.6	5.6	8	0.96	0.557	-0.24	204	7	168
4/2/96	15	C135	ClO_2		8	8.00	48.6	4.11	0.98	1.04	1	1.02	1.06	180 sec	5.5	5.5	5.5	8	0.97	0.466	-0.32	109	1	126
4/2/96	16	C135	ClO_2		8	8.00	48.6	4.11	0.98	1.15	1.09	1.12	1.18	360 sec	4.5	6.2	5.35	8	0.97	0.228	-0.63	60	1	206
4/2/96	17	C135	ClO_2		8	8.00	48.6	4.11	0.98	1.14	1.05	1.1	1.17	540 sec	4.5	6.8	5.65	8	0.97	0.121	-0.9	44	2	333
4/16/96	33	C166	ClO_2		6	6.00	60.7	3.3	0.98	0.93	0.88	0.91	0.94	4 min	5.6	5.6	5.6	6	0.94	0.712	-0.12	180	47	92
4/16/96	34	C166	ClO_2		6	6.00	60.7	3.3	0.98	0.94	0.09	0.52	0.95	8 min	4.5	5.2	4.85	6	0.94	0.519	-0.26	151	13	152
4/16/96	35	C166	ClO_2		6	6.00	60.7	3.3	0.98	0.98	0.92	0.95	1	12 min	5.6	5.8	5.7	6	0.94	0.265	-0.55	117	0	325
4/18/96	37	C173	ClO_2		6	6.00	221	0.91	0.99	1.05	0.93	0.99	1.06	24 min	4.8	5.9	5.35	6	0.97	0.050	-1.29	22	1	436
4/18/96	38	C173	ClO_2		6	6.00	221	0.91	0.99	1.04	0.9	0.97	1.05	12 min	5	5.8	5.4	6	0.97	0.156	-0.79	64	4	369
4/18/96	39	C173	ClO_2		8	8.00	221	0.91	0.99	1.05	0.99	1.02	1.06	600 sec	4.9	6.1	5.5	6	0.97	0.151	-0.81	72	2	416
5/4/96	52	D20	ClO_2		11	11.00	91.7	2.18	0.99	0.98	0.99	0.99	0.99	10 sec	5.6	5.6	5.6	11	0.91	0.114	-0.9	35	0	273
5/4/96	53	D20	ClO_2		11	11.00	91.7	2.18	0.99	1.05	1.05	1.05	1.06	5 sec	4.6	4.6	4.6	11	0.91	0.258	-0.55	69	0	198
5/4/96	54	D20	ClO_2		11	11.00	91.7	2.18	0.99	1	0.97	0.99	1.01	25 sec	4.5	4.5	4.5	11	0.91	0.029	-1.5	12	1	443
6/27/96	112	D150	ClO_2	condensation on UV cell	8	8.00	236	0.85	0.99	1	1	1	1	300 sec	6.3	5.8	6.05	8	0.94	0.150	-0.8	80	1	458
6/28/96	116	D154	ClO_2		6	5.99	246	0.81	0.99	1.04	0.98	1.01	1.05	10 min	5.6	6	5.8	8	0.98	0.148	-0.82	78	2	460
7/4/96	121	D161	ClO_2	Old Tube	11	10.97	243	0.82	0.99	1.3	1.3	1.3	1.31	5 sec	5.2	5.2	5.2	11	0.97	0.195	-0.7	109	6	476
7/4/96	122	D161	ClO_2	New Tube	11	10.97	243	0.82	0.99	1.1	1.11	1.11	1.11	5 sec	5.6	5.6	5.6	11	0.97	0.149	-0.81	95	6	579
7/4/96	123	D161	ClO_2		8	8.00	243	0.82	0.99	1.33	1.26	1.3	1.34	300 sec	5.5	7	6.25	11	0.97	0.207	-0.67	80	1	311
7/5/96	125	D166	ClO_2		11	11.04	237.4	0.84	0.99	1.06	1.06	1.06	1.07	5 sec	5.5	5.5	5.5	11	0.94	0.124	-0.88	44	1	319
3/26/96	5	C121	NH_2Cl		8	8.00	146	2.74	1.97	2	2	2	1.02	90 min	5.2	5.6	5.4	8	0.95	0.757	-0.1	64	67	42
3/26/96	6	C121	NH_2Cl		8	8.00	146	2.74	1.97	2	2	2	1.02	120 min	3.5	4.6	4.05	8	0.95	0.513	-0.27	83	81	156
3/26/96	7	C121	NH_2Cl		8	8.00	146	2.74	1.97	2	2.02	2.01	1.02	150 min	4.5	4.9	4.7	8	0.95	0.407	-0.37	73	51	181
3/26/96	8	C121	NH_2Cl		8	8.00	146	2.74	1.97	2.01	1.96	1.99	1.02	180 min	4.4	5.4	4.9	8	0.95	0.253	-0.57	65	30	280
3/26/96	8	C121	NH_2Cl		8	8.00	146	2.74	1.97	2.01	1.96	1.99	1.02	180 min	4.4	5.4	4.9	8	0.95	0.253	-0.57	65	30	280
5/9/96	56	D26	NH_2Cl		11	10.93	150.3	2.67	1.97	1.81	2.06	1.95	0.93	120 min	4.5	4.6	4.55	10.9	0.96	0.098	-0.99	18	8	239
5/9/96	58	D26	NH_2Cl		11	10.93	150.3	2.67	1.97	1.9	2.13	2.02	0.96	180 min	4.6	4.6	4.6	10.9	0.96	0.045	-1.33	23	5	601
5/15/96	71	D51	NH_2Cl		11	10.96	154	2.6	1.97	2	1.98	1.99	1.01	70 min	5.4	4.5	4.95	11	0.94	0.062	-1.18	15	13	424
5/17/96	74	D55	NH_2Cl		11	10.96	150	2.67	1.97	1.96	1.98	1.97	0.99	30 min	4.6	4.4	4.5	11	0.94	0.068	-1.14	28	12	552
5/17/96	75	D55	NH_2Cl		11	10.96	150	2.67	1.97	2.08	2.09	2.09	1.06	50 min	5	5	5	11	0.94	0.094	-1	30	17	454
5/17/96	76	D55	NH_2Cl		11	10.96	150	2.67	1.97	1.99	2.02	2.01	1.01	70 min	5	4.8	4.9	11	0.94	0.067	-1.15	20	17	513

Table C3. Single Dose Experimental Results

Date	Exp #	Lab Book page	Test	Comments	Test pH	pH	First disinfectant							Contact Time		Control				Test		Counts		
						mean	Dose Stock mg/L	Vol added mL	Applied Conc mg/L	Mean Initial mg/L	Mean Final mg/L	Mean Ave mg/L	Ratio Init /App			Temp Start °C	Temp End °C	Temp Ave °C	pH	Excystation ratio	Excystation ratio	Log Inactivation ratio	PET	ECW	IC
5/23/96	78	D59	NH_2Cl		11	10.96	165	2.42	1.97	1.87	1.86	1.87	0.95	5	min	4.3	5.4	4.85	11	0.93	0.292	-0.5	50	42	223
6/8/96	106	D106	NH_2Cl		11	10.95	134	2.99	1.97	1.83	1.85	1.84	0.93	5	min	4.9	5	4.95	8	0.89	0.111	-0.9	29	7	287
7/5/96	126	D166	NH_2Cl		11	11.04	167	2.36	1.94	1.78	1.66	1.72	0.92	5	min	5.6	5	5.3	11	0.94	0.177	-0.73	31	12	200
7/12/96	131	D173	NH_2Cl		8	7.99	149	2.68	1.97	1.98	1.95	1.97	1.01	150	min	5.8	4.6	5.2	6	0.97	0.395	-0.39	48	16	98
4/3/96	21	C141	O_3	Reject -High temp	8	8.00	24.4	1.65	0.2	0.18	0.17	0.18	0.9	15	sec	7.5	7.5	7.5	8	0.96	0.070	-1.13	23	1	317
4/3/96	22	C141	O_3		8	8.00	25.5	1.65	0.21	0.19	0.17	0.18	0.91	30	sec	5.5	5.5	5.5	8	0.96	0.171	-0.75	68	1	334
4/4/96	23	C141	O_3		8	8.00	24.9	1.65	0.2	0.19	0.16	0.18	0.93	60	sec	5.6	5.6	5.6	8	0.96	0.017	-1.74	8	1	307
4/4/96	25	C147	O_3		8	8.00	25.9	1.69	0.22	0.19	0.18	0.19	0.88	15	sec	5.4	5.9	5.65	8	0.98	0.281	-0.54	110	2	286
4/4/96	26	C147	O_3		8	8.00	25.8	1.69	0.22	0.18	0.16	0.17	0.84	30	sec	4.7	4.7	4.7	8	0.98	0.251	-0.59	76	1	239
4/4/96	27	C147	O_3		8	8.00	25.5	1.69	0.21	0.2	0.18	0.19	0.94	45	sec	5.5	5.6	5.55	8	0.98	0.110	-0.95	35	3	309
4/23/96	41	C179	O_3		6	6.00	26.3	1.66	0.22	0.15	0.15	0.15	0.7	20	sec	5.4	5.4	5.4	6	0.96	0.614	-0.19	135	94	144
4/23/96	42	C179	O_3		6	6.00	26.3	1.66	0.22	0.17	0.16	0.17	0.79	50	sec	5.2	5.2	5.2	6	0.96	0.412	-0.37	102	26	183
4/23/96	43	C179	O_3		6	6.00	26.3	1.66	0.22	0.19	0.15	0.17	0.88	90	sec	4.6	5.6	5.1	6	0.96	0.232	-0.62	65	15	265
5/1/96	45	D11	O_3		6	6.00	26.1	1.67	0.22	0.18	0.15	0.17	0.83	70	sec	4.7	6	5.35	6	0.975	0.271	-0.56	76	13	239
5/1/96	46	D11	O_3		6	6.00	26.2	1.67	0.22	0.18	0.15	0.17	0.83	180	sec	4.8	5.6	5.2	6	0.975	0.036	-1.43	12	4	428
5/1/96	47	D11	O_3		11	11.00	26.4	1.67	0.22	0.07	0	0.04	0.32	90	sec	4.5	5.4	4.95	6	0.975	0.055	-1.25	18	2	344
5/11/96	61	D37	O_3		11	10.95	26.2	1.69	0.22	0.14	0.12	0.13	0.64	10	sec	5.5	5.5	5.5	8	0.95	0.149	-0.81	42	2	252
5/11/96	62	D37	O_3		8	8.01	26.1	1.69	0.22	0.19	0.18	0.19	0.87	20	sec	4.9	4.9	4.9	8	0.95	0.129	-0.87	37	4	277
5/11/96	63	D37	O_3		11	10.95	26.7	1.69	0.22	0.15	0.09	0.12	0.68	25	sec	5.2	5.2	5.2	8	0.95	0.053	-1.26	14	1	270
5/14/96	64	D43	O_3		11	10.93	25.3	1.77	0.22	0.14	0.14	0.14	0.63	5	sec	5.5	5.5	5.5	8	0.93	0.313	-0.47	94	17	244
7/17/96	134	D176	O_3		6	6.02	20.7	2.14	0.22	0.18	0.17	0.18	0.83	60	sec	4.8	6.7	5.75	8	0.97	0.285	-0.53	83	12	238
7/17/96	135	D176	O_3		8	8.01	20.7	2.14	0.22	0.2	0.19	0.2	0.91	15	sec	6	6.4	6.2	8	0.97	0.130	-0.87	37	0	248
7/17/96	136	D176	O_3		11	11.04	220.8	2.14	0.22	0.12	0.09	0.11	0.55	5	sec	5.7	6.2	5.95	8	0.97	0.422	-0.36	92	16	148
3/24/96	4	C117	Control		8	8.00								90	min	5	5	5	8	0.95	0.955	0	55	263	15
3/26/96	9	C124	Control		8	8.00								180	min	4.5	5.8	5.15	8	0.95	0.953	0	60	104	8
3/31/96	13	C129	Control		8	8.00								180	sec				8	0.96	0.959	0	118	187	13
4/2/96	18	C135	Control		8	8.00								540	sec	4.5	5	4.75	8	0.97	0.973	0	112	144	7
4/3/96	19	C141	Control		8	8.00								60	sec	5	5	5	8	0.96	0.964	0	124	171	11
4/4/96	24	C147	Control		8	8.00								45	sec	5	5	5	8	0.98	0.978	0	85	141	5
4/11/96	28	C156	Control		6	5.98								60	min	4	4	4	6	0.95	0.948	0	130	159	16
4/14/96	32	C162	Control		6	5.96								40	min	5	5	5	6	0.95	0.954	0	173	176	17
4/16/96	36	C166	Control		6	6.00								12	min	5	5	5	6	0.94	0.938	0	124	117	16
4/18/96	40	C173	Control		6	6.00								24	min	4	4	4	6	0.97	0.975	0	171	218	10
4/23/96	44	C179	Control		6	6.00								90	sec	5	5	5	6	0.96	0.964	0	88	127	8
5/1/96	48	D11	Control		6	6.00								180	sec	5	5	5	6	0.975	0.975	0	74	121	5
5/2/96	49	D17	Control		11	11.00								180	min	5	5	5	11	0.96	0.958	0	105	124	10
5/4/96	55	D20	Control		11	11.00								25	sec	5.6	5.6	5.6	11	0.91	0.907	0	115	80	20
5/9/96	59	D26	Control		11	10.93								180	min	5	5	5	10.9	0.96	0.956	0	100	116	19
5/11/96	60	D37	Control		8	8.00								25	sec	5	5	5	8	0.95	0.950	0	92	97	10
5/14/96	69	D43	Control		8	7.98								60	min				8	0.93	0.932	0	106	100	15
5/15/96	70	D51	Control		11	10.96								70	min	5.2	5	5.1	11	0.91	0.911	0	116	157	17
5/17/96	73	D55	Control		11	10.96								70	min	5.8	5.8	5.8	11	0.91	0.940	0	83	73	10
5/23/96	77	D59	Control		11	10.96								60	min				11	0.93	0.926	0	95	93	15
5/24/96	80	D63	Control		8	8.00								210	min				8	0.94	0.942	0	74	154	11
5/28/96	85	D71	Control		6	5.99								155	min				6	0.95	0.948	0	117	118	13

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Table C3. Single Dose Experimental Results

Date	Exp #	Lab Book page	Test	Comments	Test pH	pH	First disinfectant												Control	Test		Counts		
						meas	Dose Stock mg/L	Vol added mL	Applied Conc mg/L	Mean Initial mg/L	Mean Final mg/L	Mean Ave mg/L	Ratio Inst /App	Contact Time		Temp Start °C	Temp End °C	Temp Ave °C	pH	Excystation ratio	Excystation ratio	Log Inactivation ratio	PET	ECW	IC
5/31/96	90	D83	Control		8	7.98								65	min				8	0.97	0.970	0	46	85	4
6/1/96	94	D89	Control		8	7.98								210	min				8	0.93	0.934	0	77	92	12
6/2/96	99	D99	Control		8	7.99								60	min				8	0.96	0.962	0	69	108	7
6/8/96	103	D106	Control		8	8.00								150	min				8	0.89	0.895	0	50	18	8
6/21/96	107	D135	Control		11	10.96								0	min	5	6.5	5.75	11	0.84	0.841	0	73	70	27
6/27/96	110	D150	Control		8	8.00								60	min				8	0.94	0.937	0	56	92	10
6/28/96	113	D154	Control		8	8.00								60	min				8	0.98	0.976	0	82	82	4
7/4/96	117	D161	Control		11	10.97								5	min	5.7	5.7	5.7	11	0.97	0.969	0	70	54	4
7/5/96	124	D166	Control		11	11.04								155	min				11	0.94	0.939	0	106	109	14
7/12/96	129	D173	Control		6	5.98								150	min	7	7	7	6	0.97	0.968	0	104	109	7
7/17/96	133	D176	Control		8	8.01								60	min	4.6		2.3	8	0.97	0.966	0	92	106	7

Table C4. Cyst Isolation Data

Date	Exp. #	Test pH	HP UV-VIS file #	Absorbance at 260 nm	Infection Age at Isolation, day		Additional Washes		Cyst Stock Counts							
		meas.	yy/mm/dd/#	10,000 cysts/mL	A	B	Qty	rpm	Dilution factor	A, dilution, 4 squares		B, dilution, 4 squares		A,B ave. per square	Cysts/mL	mL for 2.00E+06 cysts
										A1	A2	B1	B2			
5/24/96	81	8.00		0.007	8	9	2	1200	10	337	335	303	292	79.19	7.92E+06	0.253
5/24/96	82	10.97		0.007	8	9	2	1200	10	337	335	303	292	79.19	7.92E+06	0.253
6/1/96	95	7.98			8		2	1200	50	74	87	77	70	19.25	9.63E+06	0.208
6/1/96	96	10.96			8		2	1200	50	74	87	77	70	19.25	9.63E+06	0.208
5/28/96	88	7.98	9605283.tim	0.008	10	6	2	1200	10	227	231	207	213	54.88	5.49E+06	0.364
5/28/96	89	5.99	9605284.tim	0.008	10	6	2	1200	10	227	231	207	213	54.88	5.49E+06	0.364
5/31/96	92	7.98	9605311.tim		7		1	1200	10	266	283	279	293	70.06	7.01E+06	0.285
5/31/96	93	6.00	9605312.tim		7		1	1200	10	266	283	279	293	70.06	7.01E+06	0.285
6/28/96	114	8.00	9606292.tim	0.01	8	9	2	1200	10	261	259	267	281	66.75	6.68E+06	0.3
6/28/96	115	5.99	9606293.tim	0.01	8	9	2	1200	10	261	259	267	281	66.75	6.68E+06	0.3
5/28/96	86	10.97	9605281.tim	0.008	10	6	2	1200	10	227	231	207	213	54.88	5.49E+06	0.364
5/28/96	87	7.98	9605282.tim	0.008	10	6	2	1200	10	227	231	207	213	54.88	5.49E+06	0.364
6/1/96	97	10.96	9606011.tim		8		2	1200	50	74	87	77	70	19.25	9.63E+06	0.208
6/1/96	98	7.98	9606012.tim		8		2	1200	50	74	87	77	70	19.25	9.63E+06	0.208
7/5/96	127	11.04	9607053.tim	0.008	8	9	1	1200	10	226	209	214	206	53.44	5.34E+06	0.374
7/5/96	128	8.01	9607051.tim	0.008	8	9	1	1200	10	226	209	214	206	53.44	5.34E+06	0.374
3/31/96	10	8.00	9603311.tim		8	9	1	1400	10	258	270	208	214	59.38	5.94E+06	0.337
3/31/96	11	8.00	9603312.tim		8	9	1	1400	10	258	270	208	214	59.38	5.94E+06	0.337
3/31/96	12	8.00	9603313.tim		8	9	1	1400	10	258	270	208	214	59.38	5.94E+06	0.337
4/2/96	15	8.00	9604021.tim		6		0	1400	10	229	274	178	258	58.69	5.87E+06	0.341
4/2/96	16	8.00	9604022.tim		6		0	1400	10	229	274	178	258	58.69	5.87E+06	0.341
4/2/96	17	8.00	9604023.tim		6		0	1400	10	229	274	178	258	58.69	5.87E+06	0.341
4/16/96	33	6.00	9604161.tim		6	7	1	1200	10	307	295	349	295	77.88	7.79E+06	0.257
4/16/96	34	6.00	9604162.tim		6	7	1	1200	10	307	295	349	295	77.88	7.79E+06	0.257
4/16/96	35	6.00	9604163.tim		6	7	1	1200	10	307	295	349	295	77.88	7.79E+06	0.257
4/18/96	37	6.00	9604181.tim		8	9	1	1200	10	247	255	209	240	59.44	5.94E+06	0.336
4/18/96	38	6.00	9604182.tim		8	9	1	1200	10	247	255	209	240	59.44	5.94E+06	0.336
4/18/96	39	8.00	9604183.tim		8	9	1	1200	10	247	255	209	240	59.44	5.94E+06	0.336
5/4/96	52	11.00	9605041.tim		9	10	1	1200	10	219	244	231	218	57.00	5.70E+06	0.351
5/4/96	53	11.00	9605042.tim		9	10	1	1200	10	219	244	231	218	57.00	5.70E+06	0.351

Table C4. Cyst Isolation Data

Date	Exp. #	Test pH	HP UV-VIS file #	Absorbance at 260 nm	Infection Age at Isolation, day		Additional Washes		Cyst Stock Counts							
		meas.	yy/mm/dd/#	10,000 cysts/mL	A	B	Qty	rpm	Dilution factor	A, dilution, 4 squares		B, dilution, 4 squares		A,B ave. per square	Cysts/mL	mL for 2.00E+06 cysts
										A1	A2	B1	B2			
5/4/96	54	11.00	9605043.tim		9	10	1	1200	10	219	244	231	218	57.00	5.70E+06	0.351
6/27/96	112	8.00	lost		6	7	1	1200	10	230	241	252	228	59.44	5.94E+06	0.336
6/28/96	116	5.99	9606291.tim	0.01	8	9	2	1200	10	261	259	267	281	66.75	6.68E+06	0.3
7/4/96	121	10.97	9607044.tim	0.01	7		1	1200	10	208	232	199	212	53.19	5.32E+06	0.376
7/4/96	122	10.97	9607042.tim	0.01	7		1	1200	10	208	232	199	212	53.19	5.32E+06	0.376
7/4/96	123	8.00	9607043.tim	0.01	7		1	1200	10	208	232	199	212	53.19	5.32E+06	0.376
7/5/96	125	11.04	9607052.tim	0.008	8	9	1	1200	10	226	209	214	206	53.44	5.34E+06	0.374
3/24/96	1	8.00			8	9	1	1400	10	178	188	181	176	45.19	4.52E+06	0.443
3/24/96	2	8.00			8	9	1	1400	10	178	188	181	176	45.19	4.52E+06	0.443
3/24/96	3	8.00			8	9	1	1400	10	178	188	181	176	45.19	4.52E+06	0.443
4/11/96	29	5.98		0.011	7	8	2	1200	10	146	140	105	134	32.81	3.28E+06	0.61
4/14/96	30	5.96			9	10	1	1200	10	257	224	193	228	56.38	5.64E+06	0.355
4/14/96	31	5.96			9	10	1	1200	10	257	224	193	228	56.38	5.64E+06	0.355
5/2/96	50	11.00			8		1	1200	10	288	253	287	303	70.69	7.07E+06	0.283
5/2/96	51	11.00			8		1	1200	10	288	253	287	303	70.69	7.07E+06	0.283
5/23/96	79	8.00			6	7	2	1200	10	157	139	170	151	38.56	3.86E+06	0.519
5/31/96	91	6.00			7		1	1200	10	266	283	279	293	70.06	7.01E+06	0.285
6/21/96	108	7.99	9606212.tim		7	8	2	1200	10	134	127	112	132	31.56	3.16E+06	0.634
6/21/96	109	6.00	9606213.tim		7	8	2	1200	10	134	127	112	132	31.56	3.16E+06	0.634
6/27/96	111	8.00			6	7	1	1200	10	230	241	252	228	59.44	5.94E+06	0.336
7/12/96	130	5.98		0.011	7	9	1	1200	10	271	273	266	246	66.00	6.60E+06	0.303
3/24/96	4	8.00			8	9	1	1400	10	178	188	181	176	45.19	4.52E+06	0.443
3/26/96	9	8.00			5	6	0		20	141	172	143	120	36.00	7.20E+06	0.278
3/31/96	13	8.00			8	9	1	1400	10	258	270	208	214	59.38	5.94E+06	0.337
4/2/96	18	8.00			6		0	1400	10	229	274	178	258	58.69	5.87E+06	0.341
4/3/96	19	8.00			6	7	1	1200	10	199	181	190	193	47.69	4.77E+06	0.419
4/4/96	24	8.00		0.014	8	9			10	218	194	184	204	50.00	5.00E+06	0.4
4/11/96	28	5.98		0.011	7	8	2	1200	10	146	140	105	134	32.81	3.28E+06	0.61
4/14/96	32	5.96			9	10	1	1200	10	257	224	193	228	56.38	5.64E+06	0.355
4/16/96	36	6.00			6	7	1	1200	10	307	295	349	295	77.88	7.79E+06	0.257
4/18/96	40	6.00			8	9	1	1200	10	247	255	209	240	59.44	5.94E+06	0.336

Table C4. Cyst Isolation Data

Date	Exp. #	Test pH	HP UV-VIS file #	Absorbance at 260 nm	Infection Age at Isolation, day		Additional Washes			Cyst Stock Counts						
		meas.	yy/mm/dd/#	10,000 cysts/mL	A	B	Qty	rpm	Dilution factor	A, dilution, 4 squares		B, dilution, 4 squares		A,B ave. per square	Cysts/mL	mL for 2.00E+06 cysts
										A1	A2	B1	B2			
4/23/96	44	6.00		0.016	6	7	1	1200	10	328	308	280	314	76.88	7.69E+06	0.26
5/1/96	48	6.00		0.012	6	7	1	1200	10	220	179	200	184	48.94	4.89E+06	0.409
5/2/96	49	11.00			8		1	1200	10	288	253	287	303	70.69	7.07E+06	0.283
5/4/96	55	11.00			9	10	1	1200	10	219	244	231	218	57.00	5.70E+06	0.351
5/9/96	59	10.93			7		1	1200	10	186	187	165	176	44.63	4.46E+06	0.448
5/11/96	60	8.00		0.013	9	10	1	1200	10	199	195	164	175	45.81	4.58E+06	0.437
5/14/96	69	7.98		0.012	6		1	1200	10	298	305	279	265	71.69	7.17E+06	0.279
5/15/96	70	10.96			7		1	1200	10	261	224	221	192	56.13	5.61E+06	0.356
5/17/96	73	10.96			8	9	1	1200	10	331	313	277	280	75.06	7.51E+06	0.266
5/23/96	77	10.96			6	7	2	1200	10	157	139	170	151	38.56	3.86E+06	0.519
5/24/96	80	8.00		0.007	8	9	2	1200	10	337	335	303	292	79.19	7.92E+06	0.253
5/28/96	85	5.99		0.008	10	6	2	1200	10	227	231	207	213	54.88	5.49E+06	0.364
5/31/96	90	7.98			7		1	1200	10	266	283	279	293	70.06	7.01E+06	0.285
6/1/96	94	7.98			8		2	1200	50	74	87	77	70	19.25	9.63E+06	0.208
6/2/96	99	7.99		0.018	9	10	2	1200	10	193	206	184	201	49.00	4.90E+06	0.408
6/8/96	103	8.00			5.5	7	1	1200	10	356	418	356	461	99.44	9.94E+06	0.201
6/21/96	107	10.96	9606211.tim		7	8	2	1200	10	134	127	112	132	31.56	3.16E+06	0.634
6/27/96	110	8.00			6	7	1	1200	10	230	241	252	228	59.44	5.94E+06	0.336
6/28/96	113	8.00		0.01	8	9	2	1200	10	261	259	267	281	66.75	6.68E+06	0.3
7/4/96	117	10.97	9607041.tim	0.01	7		1	1200	10	208	232	199	212	53.19	5.32E+06	0.376
7/5/96	124	11.04		0.008	8	9	1	1200	10	226	209	214	206	53.44	5.34E+06	0.374
7/12/96	129	5.98		0.011	7	9	1	1200	10	271	273	266	246	66.00	6.60E+06	0.303
7/17/96	133	8.01		0.008	7	8	2	1200	50	87	67	51	78	17.69	8.84E+06	0.226
3/26/96	5	8.00			5	6	0		20	141	172	143	120	36.00	7.20E+06	0.278
3/26/96	6	8.00			5	6	0		20	141	172	143	120	36.00	7.20E+06	0.278
3/26/96	7	8.00			5	6	0		20	141	172	143	120	36.00	7.20E+06	0.278
3/26/96	8	8.00			5	6	0		20	141	172	143	120	36.00	7.20E+06	0.278
5/9/96	56	10.93			7		1	1200	10	186	187	165	176	44.63	4.46E+06	0.448
5/9/96	57	10.93			7		1	1200	10	186	187	165	176	44.63	4.46E+06	0.448
5/9/96	58	10.93			7		1	1200	10	186	187	165	176	44.63	4.46E+06	0.448
5/15/96	71	10.96			7		1	1200	10	261	224	221	192	56.13	5.61E+06	0.356

Table C4. Cyst Isolation Data

Date	Exp. #	Test pH	HP UV-VIS file #	Absorbance at 260 nm	Infection Age at Isolation, day		Additional Washes		Cyst Stock Counts						
										A, dilution, 4 squares		B, dilution, 4 squares		A,B ave. per square	Cysts/mL	mL for 2.00E+06 cysts
		meas.	yy/mm/dd/#	10,000 cysts/mL	A	B	Qty	rpm	Dilution factor	A1	A2	B1	B2			
5/15/96	72	10.96			7		1	1200	10	261	224	221	192	56.13	5.61E+06	0.356
5/17/96	74	10.96			8	9	1	1200	10	331	313	277	280	75.06	7.51E+06	0.266
5/17/96	75	10.96			8	9	1	1200	10	331	313	277	280	75.06	7.51E+06	0.266
5/17/96	76	10.96			8	9	1	1200	10	331	313	277	280	75.06	7.51E+06	0.266
5/23/96	78	10.96			6	7	2	1200	10	157	139	170	151	38.56	3.86E+06	0.519
6/8/96	106	10.95		0.01	5.5	7	1	1200	10	356	418	356	461	99.44	9.94E+06	0.201
7/5/96	126	11.04		0.008	8	9	1	1200	10	226	209	214	206	53.44	5.34E+06	0.374
7/12/96	131	7.99		0.011	7	9	1	1200	10	271	273	266	246	66.00	6.60E+06	0.303
7/12/96	132	7.99		0.011	7	9	1	1200	10	271	273	266	246	66.00	6.60E+06	0.303
5/14/96	65	10.93	9605144.tim	0.012	6		1	1200	10	298	305	279	265	71.69	7.17E+06	0.279
5/14/96	67	7.98	9605142.tim	0.012	6		1	1200	10	298	305	279	265	71.69	7.17E+06	0.279
5/14/96	68	6.02	9605143.tim	0.012	6		1	1200	10	298	305	279	265	71.69	7.17E+06	0.279
6/2/96	100	5.99	9606021.tim	0.018	9	10	2	1200	10	193	206	184	201	49.00	4.90E+06	0.408
6/2/96	101	7.99	9606022.tim	0.018	9	10	2	1200	10	193	206	184	201	49.00	4.90E+06	0.408
6/2/96	102	10.96	9606023.tim	0.018	9	10	2	1200	10	193	206	184	201	49.00	4.90E+06	0.408
5/24/96	83	8.00	9605242.tim	0.007	8	9	2	1200	10	337	335	303	292	79.19	7.92E+06	0.253
5/24/96	84	10.97	9605241.tim	0.007	8	9	2	1200	10	337	335	303	292	79.19	7.92E+06	0.253
6/8/96	104	8.00	9606081.tim	0.01	5.5	7	1	1200	10	356	418	356	461	99.44	9.94E+06	0.201
6/8/96	105	10.95	9606082.tim	0.01	5.5	7	1	1200	10	356	418	356	461	99.44	9.94E+06	0.201
4/3/96	21	8.00	9604031.tim	0.01	6	7	1	1200	10	199	181	190	193	47.69	4.77E+06	0.419
4/3/96	22	8.00	9604032tim	0.01	6	7	1	1200	10	199	181	190	193	47.69	4.77E+06	0.419
3/4/96	23	8.00	9604033.tim	0.01	6	7	1	1200	10	199	181	190	193	47.69	4.77E+06	0.419
4/4/96	25	8.00	9604041.tim	0.014	8	9			10	218	194	184	204	50.00	5.00E+06	0.4
4/4/96	26	8.00	9604042.tim	0.014	8	9			10	218	194	184	204	50.00	5.00E+06	0.4
4/4/96	27	8.00	9604043.tim	0.014	8	9			10	218	194	184	204	50.00	5.00E+06	0.4
4/23/96	41	6.00	9604231.tim	0.016	6	7	1	1200	10	328	308	280	314	76.88	7.69E+06	0.26
4/23/96	42	6.00	9604232.tim	0.016	6	7	1	1200	10	328	308	280	314	76.88	7.69E+06	0.26
4/23/96	43	6.00	9604233.tim	0.016	6	7	1	1200	10	328	308	280	314	76.88	7.69E+06	0.26
5/1/96	45	6.00	9605011.tim	0.012	6	7	1	1200	10	220	179	200	184	48.94	4.89E+06	0.409
5/1/96	46	6.00	9605012.tim	0.012	6	7	1	1200	10	220	179	200	184	48.94	4.89E+06	0.409
5/1/96	47	11.00	9605013.tim	0.012	6	7	1	1200	10	220	179	200	184	48.94	4.89E+06	0.409

Table C4. Cyst Isolation Data

Date	Exp. #	Test pH	HP UV-VIS file #	Absorbance at 260 nm	Infection Age at Isolation, day		Additional Washes		Cyst Stock Counts							
		meas.	yy/mm/dd/#	10,000 cysts mL	A	B	Qty	rpm	Dilution factor	A, dilution, 4 squares		B, dilution, 4 squares		A,B ave. per square	Cysts/mL	mL for 2.00E+06 cysts
										A1	A2	B1	B2			
5/11/96	61	10.95	9605111.tim	0.013	9	10	1	1200	10	199	195	164	175	45.81	4.58E+06	0.437
5/11/96	62	8.01	9605113.tim	0.013	9	10	1	1200	10	199	195	164	175	45.81	4.58E+06	0.437
5/11/96	63	10.95	9605112.tim	0.013	9	10	1	1200	10	199	195	164	175	45.81	4.58E+06	0.437
5/14/96	64	10.93	9605141.tim	0.012	6		1	1200	10	298	305	279	265	71.69	7.17E+06	0.279
7/17/96	134	6.02	9607191.tim	0.008	7	8	2	1200	50	87	67	51	78	17.69	8.84E+06	0.226
7/17/96	135	8.01	9607192.tim	0.008	7	8	2	1200	50	87	67	51	78	17.69	8.84E+06	0.226
7/17/96	136	11.04	9607193.tim	0.008	7	8	2	1200	50	87	67	51	78	17.69	8.84E+06	0.226